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TRANSLOCATION OF NITROGENOUS SUBSTANCES IN THE CUTHBERT RASPBERRY[1]

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(WITH THREE FIGURES)

Introduction

In studies dealing with the translocation of nitrogenous compounds in plants, there seems to have been a tendency to search for the same plan of movement which has been observed for carbohydrates. It is apparent, however, that much of the translocation of nitrogen will differ markedly from that of carbohydrates, because the places of syntheses, unlike photosynthesis, may be found in almost any or all parts of the plant (**6, 18, 16, 17, 19, 20, 21, 8**).

This paper presents the results of a study of the translocation and metabolism of the nitrogenous substances of the Cuthbert raspberry, and correlates these with the results of a study of carbohydrates obtained at the same time and already reported (**7**).

Recent literature dealing with nitrogen metabolism of plants has been reviewed by NIGHTINGALE (**15**) and MCKEE (**13**). The translocation of nitrogenous substances has been discussed by CLEMENTS (**1**), CURTIS (**4**), and MASON and PHILLIS (**12**).

Material and methods

The Cuthbert raspberry, a hybrid of *Rubus strigosus* × *R. idaeus*, each year produces tall, erect, biennial, vegetative canes from peren-

[1] Contribution no. 58 from the botany department of the State College of Washington.

nial roots. These vegetative canes develop all of their height and most of their diameter during the first season, and in the second season bear fruit on short lateral branches. The studies here reported refer only to the first year or vegetative plants.

TREATMENT AND SAMPLING.—Double rings were used to check movements of materials in the bark, one 15 cm. and another 30 cm. above the ground, thereby isolating a 15 cm. segment of bark between the rings. For analysis the canes were cut at the rings and the portion of the cane above the rings was cut into 15 cm. lengths. The control plants likewise were cut for analysis into 15 cm. lengths, measuring from the ground upward; the second segment of the control thus corresponds with the isolated segment (between the rings) of the treated plant.

Canes were selected in three groups of twelve plants each. The canes of the first group were removed by cutting at the ground level, and these served as controls for the beginning of the experiment. The stems of the second group were ringed in the manner described. The canes of the third group were tagged (but not ringed) and left to be removed as controls at the time of the next ringing treatment. The first treatment and collection of controls were made on May 15. The ringed plants and the tagged plants were harvested on June 23. Thus the tagged plants served as end controls for the May 15 series and as beginning controls for the June 23 series. More canes were ringed and others tagged to be left as controls on June 23. These were harvested on July 15, and the procedure repeated. The plants ringed and those tagged for end controls on July 15 were collected on August 9. These were the end controls for the whole seasonal study.

MOISTURE.—The moisture content of the canes employed was assumed to be the same as that obtained for the similar canes used in the carbohydrate studies.

NITROGEN DETERMINATION.[2]—The fresh plant material, which had been autoclaved at 15 lb. pressure for 15 minutes, was ground in a plate grinder with the addition of generous quantities of water. This mixture was brought to a boil, and filtered with suction after

[2] Unless otherwise specified, the term nitrogen is used in this paper as an abbreviation of the term nitrogenous compounds.

the addition of 10 cc. of 10 per cent acetic acid to coagulate the colloidal nitrogen. The residue after several washings was discarded. The filtrate was diluted to a volume of 2 liters, and this was the stock solution from which aliquots were taken for determination of the various fractions. The procedure of fractionation was that reported by LEONARD (8).

EXPRESSION OF RESULTS.—The percentages of nitrogen, as well as the absolute amounts, are given for each segment of stem, and for the leaves of the segment. The latter were analyzed separately from the stem segment. For convenience in reporting the results, the segments of stem were designated, from the ground upward, as 1st, 2st, 3st, etc.; the corresponding leaves were designated as 1L, 2L, 3L, etc. Thus in the ringed plants 1st is that segment of stem below the lower ring, 2st is that between the rings, and 3st is that just above the upper ring.

Investigation

DISTRIBUTION OF NITROGENOUS SUBSTANCES IN NORMAL PLANTS, AND EFFECT OF RINGING

TOTAL ORGANIC NITROGEN.—Within the cane the highest percentages of total organic nitrogen are found in the topmost stem segment and leaves, with a downward gradient in the stem to the lowest concentration in the basal segment. This might be expected in view of the meristematic tissues present in the tip in contrast to the senescent tissues at the base. The percentage figures of total organic nitrogen are little more than indices of the proportion of living, protoplasm-containing cells (meristems, cambiums, parenchyma) to non-living, non-protoplasmic cells with thickened walls (tracheids, vessels, sclerenchyma). In general, as the plants age with the progress of summer, cell wall and secondary thickening continue, residual carbohydrates increase, while a considerable portion of the protoplasm disappears and the percentages of total organic nitrogen decrease (tables 1, 2; figs. 1, 2).

In all segments of the stem above the lower ring, including the isolated segment, the loss of nitrogen (percentage of residual dry weight) is not so great as in the normal stems; that is, there is more organic nitrogen per gram of residual dry weight left at the end of

the period of treatment in the ringed than in the normal stems. Below the lower ring, in the basal segment, loss of organic nitrogen is greater than normal. These results are caused by the accumulation of carbohydrates and other substances above the ring, which are effective in producing callus growth as well as accelerated growth in diameter of the region above the rings. The increased growth means in turn increased amounts of protoplasm, with its abundance of nitrogen. Relative to the residual polysaccharides in the stem,

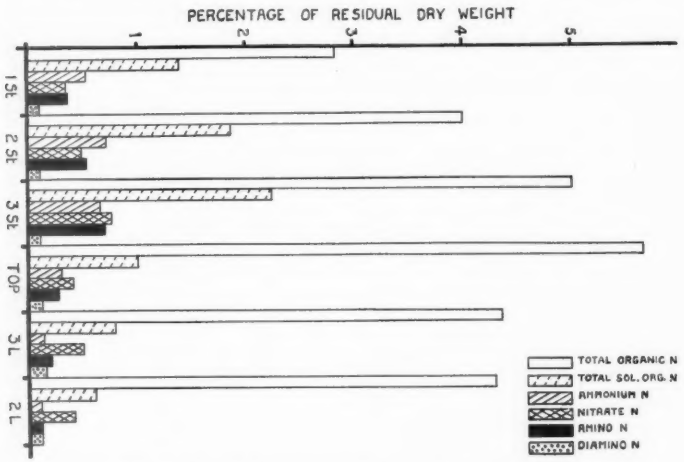

FIG. 1.—Distribution of nitrogenous substances in normal plants; May 15

the percentage of the latter is greater. Thus ringing results in a higher organic nitrogen content of the stem immediately above the rings by increasing the amount of living tissue, but this effect is manifested by a smaller percentage loss of nitrogen above the rings as the summer progresses. There is a loss of total organic nitrogen below the rings, owing to the apparent prevention of growth of the basal stem segment. The effect of the rings in preventing the downward movement of carbohydrates may be partly responsible for the inhibited growth; but since carbohydrate does move into the basal segment from the roots (7), the effect may be more directly a result of the prevention of downward movement of growth regulators.

The leaves of the June plants developed, with the exception of those growing on segment 3st, after the rings were made on May 15.

The organic nitrogen content of these leaves was much lower than that of the leaves which developed on the normal plants during the

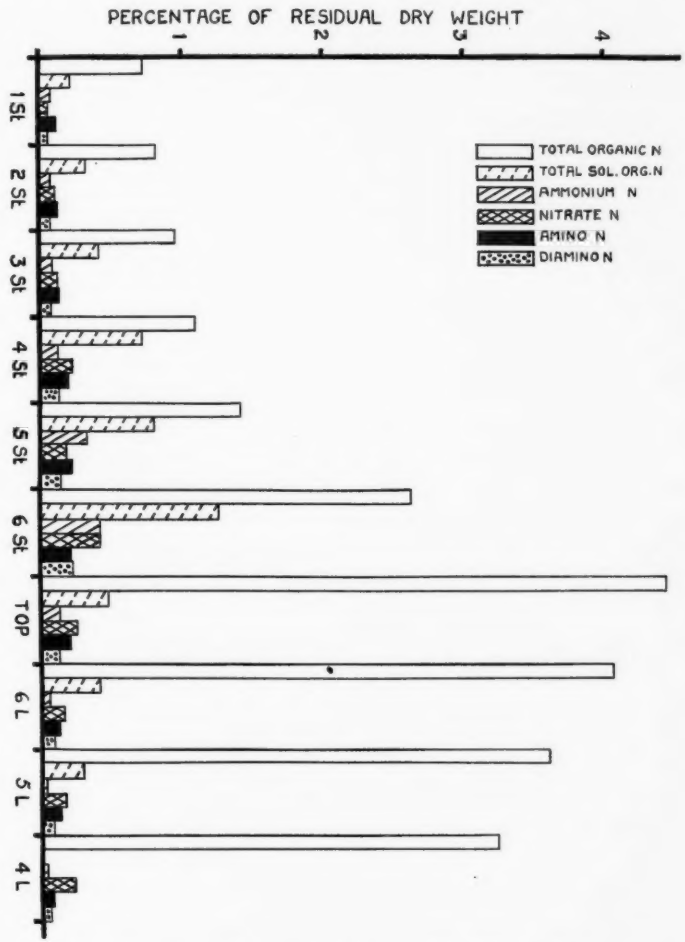

FIG. 2.—Distribution of nitrogenous substances in normal plants; June 23

same time, indicating that ringing had in some way inhibited the normal formation of protein in the leaves, possibly by disruption of water relations. The leaves of the plants ringed on June 23 and

gathered on July 16 showed considerable reduction in percentage of organic nitrogen, whereas the normal plants showed an increase in percentage of total organic nitrogen. The leaves of the treated plants of the August collection, however, showed substantial increases over the normal plants. These were ringed on July 16, when the plants were mature, and the treatment apparently caused less injury than it did on the plants collected in June and July.

When the plants were ringed on May 15 they consisted of three segments and the tops; that is, they were approximately 50 cm. tall. When they were harvested on June 23 they were six segments (plus tops) tall, about 100 cm. By June 23 they had made approximately twice the growth in height, while the dry weight of the stem above the upper rings increased from 20.9 gm. at the time of ringing on May 15 to 72.4 gm. by June 23. The dry weight of the corresponding portion of the stem of the normal plants increased from 20.9 gm. on May 15 to 63.5 gm. on June 23. The plants harvested on July 16 had not increased so greatly in height over their beginning controls (June 23 normal canes) as had those of June. Although data for the uppermost segment and tops are not available, a comparison of the remaining segments of the normal plants of July 16 with those of June 23 reveals that there was a small loss of organic nitrogen per segment in grams and in percentage of residual dry weight, whereas from May 15 to June 23 there was considerable loss from each segment. The ringed canes collected on July 16 showed a slight increase in organic nitrogen over the beginning controls collected on June 23. Similar relationships obtained in the August plants.

Three factors are operative in causing a decrease in nitrogen per segment in the normal and in the ringed plants. The first of these factors is essentially a "dilution," brought about by increasing cell wall thickening and by the production of secondary tissue in which thick-walled xylem is most abundant. The second factor is the large amount of secondary xylem produced in May and June when differentiation of vessels and tracheids results in a loss of protoplasm. The nitrogenous constituents of the protoplasm, not entering into secondary wall formation, perhaps move to the rapidly growing upper portion of the plant. The third factor concerns the loss of

nitrogen from the stem, either by migration to the roots or directly to the environment.

Ringing interferes with the carbohydrate status of the stem by causing a large accumulation above the rings. Where carbohydrates accumulate so also do nitrogenous compounds if inorganic nitrogen is available. There appears to be considerable synthesis of organic nitrogen throughout the whole length of the stem, since growth continues and is usually stimulated by the abundance of carbohydrates. Thus above the rings the dilution factor is even more important than it is in unringed canes. Accumulation of nitrogen by a carbohydrate-gorged stem, and an increased amount of protoplasm owing to accelerated growth, operate against the loss of nitrogenous substances by dilution through cell wall thickening and loss of protoplasm incidental to the differentiation of tracheae, tracheids, and fibers. From May 15 to June 23 percentage loss of organic nitrogen in the ringed plants was greater than accumulation, but the net loss was smaller than that of normal plants during the same period. From June 23 to July 16, and from July 16 to August 9, loss in percentage of organic nitrogen in the ringed segments was less than the accumulation of nitrogen, owing to the abundance of carbohydrates; and the organic nitrogen concentration was greater than that of the beginning controls of each group. The normal plants also lost organic nitrogen through the factors previously mentioned. Thus when dealing with amount of organic nitrogen per unit of any maturing or differentiating tissue, the normal process is to lose organic nitrogen; when accumulations result through any means in a maturing or differentiating tissue, the accumulation is a net result which includes loss through thickening and differentiation. In this paper the term accumulation is used in that sense, and its criterion is a concentration higher than that of the normal plants harvested at the same time. For example, in the ringed plants harvested in June the concentration of organic nitrogen is higher than that of the normal plants taken at the same time, but the concentration in each is lower than that of its beginning control, the normal plants of May 15.

SOLUBLE ORGANIC NITROGEN AND PROTEIN.—Concentration of the soluble forms of organic nitrogen increases in the stem from the

basal to the subterminal segment, where occurs the highest concentration of all regions of the entire plant (tables 1, 2; figs. 1, 2). Concentration of this composite fraction in the tops and in the leaves is more than 50 per cent lower than in the subterminal stem segment. Since the concentration of total organic nitrogen was highest in the tops (the portion including the stem tip and small unfolding leaves), it appears that concentration of the protein fraction, obtained by subtracting the soluble nitrogen from the total organic nitrogen,[3] was highest in the stem tip and small leaves where the tissues are little differentiated and mostly parenchymatous. The young leaves are largely meristematic, and the amount of protoplasm per unit of residual dry weight is higher in this than in any other region of the plant. Thus the content of soluble nitrogen is low in the leaves and stem tip, while the protein content is high. This seems to indicate rapid utilization of the soluble forms in the synthesis of protein in this region of great meristematic activity.

Soluble nitrogen, like the total organic, showed a gradual decrease in concentration per segment with the progress of summer, brought about by the factors already discussed. A similar decrease in soluble nitrogen from an early season high was noted by LEONARD (**8**) in the sunflower.

Below the lower ring in the treated plants there was an accumulation of soluble organic nitrogen which was twice that of the corresponding segments of the control. In the treated plants of July the accumulation not only doubled the amount of soluble organic nitrogen in the check plants taken at the same time, but was greater than that of the beginning controls harvested on June 23. There was no such accumulation in the more mature August plants.

Between the rings the percentage of soluble organic nitrogen was above the normal (fig. 3). The value for this segment is normally intermediate between that of the first and third segments in the untreated plants. The total organic nitrogen, which includes protein, is intermediate in concentration in the segment between the rings. This indicates that proteolysis occurred to some extent, increasing the amounts of the soluble forms, particularly alpha amino acids

[3] The figures for protein are at best only rough estimates, and should be interpreted accordingly.

and ammonium nitrogen, with a corresponding loss of protein (fig. 3). A similar accumulation of nitrogen appeared in the ringed plants of the July and August collections.

Above the rings the concentration of soluble organic forms remained approximately normal in all series. The concentrations in the leaves of the ringed plants collected in June were lower than normal, possibly owing to inhibition of the growth rate by ringing so early in the life of the plant. The fact that there was a larger amount of soluble organic nitrogen in the stem above the rings than in the normal stem is significant, because when coupled with the increase of total organic nitrogen above normal it means that protein synthesis continued beyond the normal rate, owing to an abundance of carbohydrates. Apparently reduction of nitrates and their change to amino acids and protein take place all along the stem, the amount of reduction being proportional to the number of living cells per unit of tissue. The nitrogen supply to this region is continuous and therefore must come from the xylem. The rings do not seriously interfere with this nitrogen supply, although accumulation of the soluble fraction below the lower ring in the June and July collections suggests interference with upward movement from the roots.

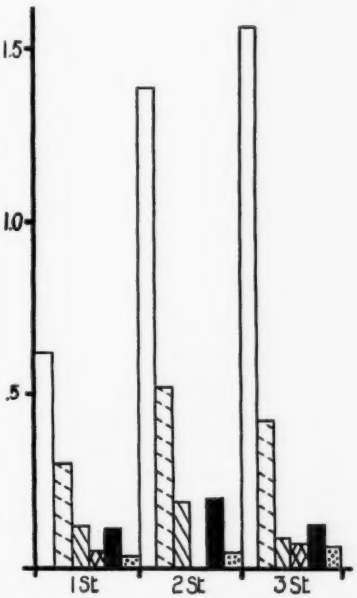

FIG. 3.—Effect of ringing on nitrogen fractions in first three segments of stem; June 23.

The total organic nitrogen concentration was lower than normal in the basal segment of the ringed plants collected in June and July, but the total soluble forms were higher, indicating that although

soluble forms accumulated below the ring, the cessation of diameter growth and partial proteolysis resulted in a loss of protein which itself probably contributed to the accumulation of soluble forms. Ringing the mature plant in July did not affect the concentration of total organic and total soluble organic nitrogen fractions in the same way, because there was an accumulation of total organic and a decrease in soluble organic nitrogen in the basal segment. This points to the likelihood of proteolysis contributing to the accumulation of soluble nitrogen rather than to interference with upward movement. The data indicate the existence of a reversible equilibrium between the soluble organic and the insoluble (protein) nitrogen.

ALPHA AMINO ACIDS.—The soluble organic fractions which are present in significant quantities are the alpha amino acids and the diamino (basic) nitrogenous compounds. Of these the alpha amino acids are more important, judging from their reactions to treatment and their closer relationship to the total soluble fraction and the proteins.

The concentration of this fraction was higher in the beginning control plants collected May 15 than in the plants of any other collection. There was a steady though not rapid decline in concentrations of alpha amino acids to the end of the season. The large concentrations in May were present during the period of most rapid growth,[4] when protein formation necessitates rapid reduction of nitrate and its ultimate transformation to amino acids. The steady loss in amounts of amino acids throughout the summer was produced not only by the factors of dilution, loss of protoplasm, and direct loss of nitrogen to the roots or to the environment, but also by a declining absorption of nitrate from soil whose nitrate supply is normally diminished by the middle of summer. In the August series the alpha amino and diamino acids constituted most of the total soluble nitrogen.

Within the normal plant the concentrations of alpha amino acids increased from the basal to the subterminal segment, in which was found the largest amount of alpha amino acids in the plant. This

[4] During the 5½ weeks between the May 15 and June 23 collections, the canes grew about 50 cm. in length ("tops" usually not 15 cm. in length).

fraction was abundant in the May stems (table 1; fig. 1), and was a prominent contributor to the soluble nitrogen peak in the subterminal segment. The concentration of alpha amino acids in the stem tip part-segment was more than 50 per cent lower than that of the subterminal segment. The leaves were 50 per cent lower in alpha amino acid content than their corresponding stem segments. As the season progressed the stem and leaf concentrations approached each other more closely.

It was pointed out previously that where concentration of the protein fraction was greatest, total soluble fraction was lowest (stem tip); and that just below the stem tip, in the subterminal segment, concentration of the soluble forms was considerably greater, with protein correspondingly lower, than it was in the stem tip. Since alpha amino acids contribute most to this difference in concentration of soluble forms, the same relationships hold.

In the ringed plants of the June and July collections there was an accumulation of alpha amino nitrogen in the basal segment, in the segment (2st) between the rings, and in the segment (3st) just above the rings (table 4). The concentration of this fraction in the ringed plants of the August collection remained nearly normal in segments 1st and 3st, but there was considerably more alpha amino nitrogen between the rings. Inhibiting the upward movement of amino acids from the roots or the downward movement from the upper regions might have produced the accumulation above and below the ring, but this could not account for the accumulation between the rings. Figure 3 shows, without reference to the controls, the distribution of total organic nitrogen and the fractions in the first three segments of the ringed canes of June. It is seen that the total organic nitrogen, which increased only slightly over the control segment, is on the graph nearly in its normal position; that is, it is intermediate between that of the first and that of the third segment. The total soluble fraction, however, increased well beyond its intermediate position, and consequently protein decreased. The increase in total soluble nitrogen, including mostly an increase in alpha amino acids, occurred at the expense of the protein. Thus it seems that the accumulation of alpha amino acids in the segment

between the rings was a result of proteolysis, and this is borne out by the external appearance of this isolated segment.

The segment above the ring had an abundance of sugars, and was actually stimulated to greater diameter growth. Considerable callus rapidly developed, with the incidental widespread meristematic activity. Synthesis of amino acids progressed at a rapid rate and there was a great increase in total organic nitrogen, much of which was protein. The accumulation above the ring therefore appeared to be produced by the synthesis of amino acids and protein, rather than by a stopping of downward movement.

The accumulation of alpha amino acids below the rings is partly reflected in the accumulation of the total soluble fraction. The total organic nitrogen value was lower than normal, and this (with the accumulation of soluble forms) points to loss of protein. It is possible that some amino acids moved in from the roots. This segment, not so senescent as that between the rings, did not increase in diameter; in fact, its cambial activity is normally very weak. A small amount of sugar moved into this segment from the roots, especially in the spring (7), and the segment lives even though the important influx of carbohydrates from above has been prevented by ringing. Ringing later in the life of the plant had no such effect on the basal segment.

DIAMINO ACIDS.—The diamino or basic nitrogen fraction contains such acids as proline, lysine, etc. The concentrations in the stems increase very gradually up to an amount equal to and often larger than the alpha amino acids in the plants of the August collection. The quantities of diamino acids in the leaves of all plants are small and often negligible (tables 1, 4).

In the stems and leaves above the rings the concentration of this fraction was slightly lower than normal. Like the alpha amino, the diamino acids accumulated in small amounts below the rings in the plants of the June collection, but there was no accumulation below the rings in the plants collected in July. In the treated plants of the August collection the concentration of this fraction was lower than normal. There were no significant differences between the concentrations in the second segment of the normal and ringed plants.

The concentrations of diamino acids seem to have some relationship to those of alpha amino acids.

AMIDES.—The concentrations of the amide fraction were insignificant (tables 1, 5).

TABLE 1

DISTRIBUTION OF NITROGENOUS SUBSTANCES IN MAY 15 CONTROLS

CANE SEGMENT	GM.	PERCENTAGE RESIDUAL DRY WEIGHT	GM.	PERCENTAGE RESIDUAL DRY WEIGHT	GM.	PERCENTAGE RESIDUAL DRY WEIGHT	GM.	PERCENTAGE RESIDUAL DRY WEIGHT
	TOTAL ORGANIC NITROGEN		SOLUBLE ORGANIC NITROGEN		AMMONIUM NITROGEN		NITRATE NITROGEN	
1st	0.665	2.82	0.3280	1.391	0.1224	0.520	0.0826	0.350
2st	0.645	3.99	0.3020	1.855	0.1164	0.716	0.0781	0.488
3st	0.415	5.07	0.1840	2.253	0.0540	0.662	0.0620	0.760
Top	0.424	5.68	0.0740	0.988	0.0208	0.279	0.0288	0.386
3L	1.500	4.37	0.2720	0.792	0.0432	0.126	0.0864	0.252
2L	1.461	4.30	.....	.....	0.0400	0.118	0.1300	0.383
	ALPHA AMINO NITROGEN		DIAMINO NITROGEN		AMIDE NITROGEN			
1st	0.0858	0.364	0.0240	0.102	0.0000	0.000		
2st	0.0844	0.519	0.0168	0.103	0.0042	0.026		
3st	0.0583	0.715	0.0092	0.112	0.0023	0.028		
Top	0.0199	0.267	0.0091	0.122	0.0008	0.011		
3L	0.0610	0.178	0.0512	0.149	0.0025	0.007		
2L	0.0425	0.125	0.0400	0.118	0.0014	0.006		

NITRATES.—This fraction, which includes nitrates and nitrites, was abundant throughout the entire plant early in the season (tables 1, 3). Its concentration in the leaves and stem tip of the May, June, and July series was nearly as high as in the stems. The concentration was very low in stem and leaves of the plants collected in August, but nitrates were supplied to the tops of the plants, for ammonium nitrogen was always present, together with amino acids, even when nitrates were very low. Continuous reduction utilizes much of the nitrate reaching the top of the plant. There is no doubt that nitrates were absorbed in greater quantities in the

TABLE 2

DISTRIBUTION OF TOTAL ORGANIC AND TOTAL SOLUBLE ORGANIC NITROGEN IN NORMAL AND RINGED CANES

Fraction	Cane segment	June 23				July 16				August 9			
		Controls		Ringed		Controls		Ringed		Controls		Ringed	
		Gm.	Percentage residual dry weight	Gm.	Percentage control residual dry weight	Gm.	Percentage residual dry weight	Gm.	Percentage control residual dry weight	Gm.	Percentage residual dry weight	Gm.	Percentage control residual dry weight
Total organic nitrogen	1st	0.1590	0.707	0.1390	0.618	0.2420	1.045	0.1255	0.541	0.1349	0.589	0.1925	0.834
	2st	0.1300	0.807	0.2240	1.390	0.1293	0.746	0.1355	0.784	0.1210	0.673	0.1785	0.992
	3st	0.1295	0.933	0.2180	1.570	0.1293	0.890	0.1440	1.000	0.1216	0.746	0.2010	1.234
	4st	0.1165	1.068	0.2150	1.973	0.1040	1.067	0.1790	1.846	0.1000	0.765	0.1774	1.363
	5st	0.1160	1.400	0.1445	1.810	0.1041	1.300	0.1015	1.270	0.0804	0.811	0.1198	1.222
	6st	0.1075	2.615	0.1152	2.810	.....	.....	.....	.....	0.1688	0.885	0.1300	1.668
	7st	.....	.....	.....	.....	.....	.....	.....	.....	0.0590	0.944	0.1180	1.870
	Top	0.5670	4.400	0.4580	3.550	.....	.....	.....	.....	0.5100	3.360	0.4380	2.880
	7L	.....	.....	.....	.....	.....	.....	.....	.....	0.4770	2.935	0.6050	3.710
	6L	0.6790	4.020	0.4840	2.865	.....	.....	.....	.....	0.4880	2.800	0.5630	3.230
	5L	0.7280	3.570	0.4910	2.410	0.6540	3.270	0.3210	1.603	0.4260	.....	0.2940	.....
	4L	0.6130	3.210	0.4600	2.425	0.4930	2.762	0.2175	1.222	.....	.....	.....	.....
	3L	0.4280	2.580	0.4070	2.455	0.4825	2.360	.....	.....	.....	.....	.....	.....

TABLE 2—*Continued*

Fraction	Cane segment	June 23				July 16				August 9			
		Controls		Ringed		Controls		Ringed		Controls		Ringed	
		Gm.	Percentage residual dry weight	Gm.	Percentage control residual dry weight	Gm.	Percentage residual dry weight	Gm.	Percentage control residual dry weight	Gm.	Percentage residual dry weight	Gm.	Percentage control residual dry weight
Total soluble organic nitrogen	1st	0.0397	0.176	0.0768	0.342	0.0257	0.111	0.0557	0.240	0.0660	0.289	0.0448	0.195
	2st	0.0576	0.356	0.0840	0.522	.....	.....	0.0768	0.444	0.0510	0.284	0.0640	0.356
	3st	0.0525	0.378	0.0548	0.420	0.0472	0.328	0.0383	0.266	0.0447	0.274	0.0384	0.236
	4st	0.0749	0.684	0.0608	0.553	0.0597	0.616	.....	.....	0.0510	0.390	0.0448	0.344
	5st	0.0640	0.800	.....	.....	0.0597	0.746	0.0426	0.533	0.0480	0.487	0.0256	0.261
	6st	0.0512	1.248	0.0450	1.100	.....	.....	.....	.....	0.0447	0.574	0.0384	0.493
	7st	.....	.....	.....	.....	.....	.....	.....	.....	0.0447	0.709	0.0725	1.150
	Top	0.0832	0.453	0.0650	0.504	.....	.....	.....	.....	0.0704	0.463	0.0575	0.378
	7L	.....	.....	.....	.....	.....	.....	.....	.....	0.0576	0.353	0.0575	0.353
	6L	0.0640	0.379	0.0450	0.266	.....	.....	.....	.....	0.0545	0.334	0.0640	0.368
	5L	0.0768	0.377	0.0480	0.235	0.0425	0.214	0.0423	0.211	0.0576	.....	0.0348	.....
	4L	.....	.....	0.0608	0.319	0.0469	0.264	.....	.....	0.0545	0.216	.....	.....
	3L	0.0576	0.347	0.0400	0.242	0.0512	0.251	.....	.....	.....	.....	.....	.....

TABLE 3

DISTRIBUTION OF ALPHA AMINO AND DIAMINO NITROGEN IN NORMAL AND RINGED CANES

Fraction	Cane segment	June 23				July 16				August 9			
		Controls		Ringed		Controls		Ringed		Controls		Ringed	
		Gm.	Percentage residual dry weight	Gm.	Percentage control residual dry weight	Gm.	Percentage residual dry weight	Gm.	Percentage control residual dry weight	Gm.	Percentage residual dry weight	Gm.	Percentage control residual dry weight
Alpha amino nitrogen	1st	0.0099	0.088	0.0203	0.090	0.0046	0.020	0.0083	0.036	0.0181	0.079	0.0166	0.073
	2st	0.0152	0.094	0.0318	0.197	0.0079	0.045	0.0134	0.078	0.0171	0.095	0.0266	0.148
	3st	0.0137	0.098	0.0171	0.123	0.0104	0.072	0.0106	0.074	0.0146	0.090	0.0146	0.089
	4st	0.0180	0.164	0.0092	0.084	0.0111	0.114	0.0090	0.094	0.0146	0.111	0.0120	0.093
	5st	0.0164	0.206	0.0081	0.101	0.0124	0.155	0.0039	0.048	0.0165	0.168	0.0070	0.072
	6st	0.0076	0.187	0.0193	0.472	.....	.....	.....	.....	.....	.....	0.0055	0.071
	7st	.....	.....	.....	.....	.....	.....	.....	.....	0.0086	0.137	.....	.....
	Top	0.0209	0.162	0.0247	0.191	0.0184	.....	0.0214	.....	0.0190	0.125	0.0206	0.135
	7L	.....	.....	.....	.....	.....	.....	.....	.....	0.0196	0.120	0.0156	0.096
	6L	0.0170	0.100	0.0231	0.136	0.0092	.....	.....	.....	0.0201	0.113	0.0105	0.061
	5L	0.0211	0.103	0.0212	0.104	0.0164	0.082	0.0139	0.069	0.0316	.....	0.0191	.....
	4L	0.0104	0.054	0.0130	0.068	0.0145	0.081	0.0130	0.074	0.0266	0.106	.....	.....
	3L	0.0118	0.071	0.0124	0.074	0.0099	0.049	.....	.....	.....	.....	.....	.....

TABLE 3—*Continued*

Fraction	Cane segment	June 23				July 16				August 9			
		Controls		Ringed		Controls		Ringed		Controls		Ringed	
		Gm.	Percentage residual dry weight	Gm.	Percentage control residual dry weight	Gm.	Percentage residual dry weight	Gm.	Percentage control residual dry weight	Gm.	Percentage residual dry weight	Gm.	Percentage control residual dry weight
Diamino nitrogen	1st	0.0048	0.022	0.0065	0.058	0.0085	0.037	0.0086	0.037	0.0176	0.077	0.0048	0.029
	2st	0.0067	0.041	0.0060	0.037	0.0107	0.062	0.0106	0.061	0.0192	0.106	0.0120	0.067
	3st	0.0051	0.036	0.0084	0.060	0.0085	0.059	0.0074	0.051	0.0160	0.098	0.0096	0.059
	4st	0.0115	0.105	0.0089	0.081	0.0107	0.109	0.0148	0.153	0.0144	0.110	0.0096	0.074
	5st	0.0088	0.110	0.0074	0.092	0.0171	0.213	0.0149	0.186	0.0160	0.162	0.0032	0.033
	6st	0.0080	0.196	0.0080	0.195	.....	.....	.....	.....	0.0160	0.206	0.0144	0.185
	7st	.....	.....	.....	.....	.....	.....	.....	.....	0.0160	0.254	0.0181	0.287
	Top	0.0128	0.099	0.0048	0.037	0.0075	.....	0.0130	.....	0.0192	0.126	0.0128	0.084
	7L	.....	.....	.....	.....	.....	.....	.....	.....	0.0160	0.098	0.0128	0.079
	6L	0.0096	0.057	0.0072	0.042	0.0085	.....	.....	.....	0.0128	0.072	0.0112	0.064
	5L	0.0112	0.055	0.0056	0.027	0.0107	0.054	0.0106	0.053	0.0128	.....	0.0112	.....
	4L	0.0088	0.046	0.0112	0.058	0.0107	0.060	0.0107	0.060	0.0144	0.057	.....	.....
	3L	0.0160	0.096	0.0056	0.033	0.0064	0.031	.....	.....	.....	.....	.....	.....

TABLE 4

DISTRIBUTION OF AMMONIUM AND NITRATE ($+NO_2$) NITROGEN IN NORMAL AND RINGED CANES

Fraction	Cane segment	June 23				July 16				August 9			
		Controls		Ringed		Controls		Ringed		Controls		Ringed	
		Gm.	Percentage residual dry weight	Gm.	Percentage control residual dry weight	Gm	Percentage residual dry weight	Gm.	Percentage control residual dry weight	Gm.	Percentage residual dry weight	Gm.	Percentage control residual dry weight
Ammonium nitrogen	1st	0.0096	0.042	0.0276	0.122	.....	.....	0.0236	0.101	0.0064	0.022	0.0043	0.018
	2st	0.0091	0.056	0.0300	0.186	.....	.....	0.0265	0.153	0.0032	0.017	0.0182	0.101
	3st	0.0088	0.063	0.0113	0.081	0.0106	0.073	0.0021	0.014	0.0048	0.029	0.0023	0.014
	4st	0.0107	0.098	0.0104	0.095	0.0064	0.065	0.0096	0.099	0.0032	0.024	0.0048	0.036
	5st	0.0235	0.291	0.0074	0.092	0.0043	0.053	0.0055	0.068	0.0016	0.016	0.0032	0.032
	6st	0.0160	0.393	0.0080	0.195	.....	.....	.....	.....	0.0032	0.041	0.0040	0.051
	7st	.....	.....	.....	.....	.....	.....	.....	.....	0.0048	0.077	0.0207	0.032
	Top	0.0128	0.099	0.0089	0.069	.....	.....	.....	.....	0.0064	.....	0.0032	0.021
	7L	.....	.....	.....	.....	.....	.....	.....	.....	0.0056	0.031	0.0056	0.034
	6L	0.0040	0.023	0.0050	0.029	.....	.....	.....	.....	0.0056	.....	0.0051	0.029
	5L	0.0056	0.027	0.0048	0.023	0.0000	0.000	0.0000	0.000	0.0064	0.025	0.0040	.....
	4L	0.0032	0.016	0.0024	0.012	0.0021	0.010	0.0009	0.005	.....	.....	.....	.....
	3L	0.0043	0.025	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

TABLE 4—*Continued*

Fraction	Cane segment	June 23				July 16				August 9			
		Controls		Ringed		Controls		Ringed		Controls		Ringed	
		Gm.	Percentage residual dry weight	Gm.	Percentage control residual dry weight	Gm.	Percentage residual dry weight	Gm.	Percentage control residual dry weight	Gm.	Percentage residual dry weight	Gm.	Percentage control residual dry weight
Nitrate and nitrite nitrogen	1st	0.0080	0.035	0.0110	0.048	0.0085	0.036	0.0108	0.046	0.0016	0.007	0.0043	0.018
	2st	0.0135	0.083	0.0003	0.001	0.0181	0.105	0.0181	0.105	0.0080	0.044	0.0042	0.023
	3st	0.0139	0.100	0.0094	0.067	0.0149	0.103	.....	.....	0.0048	0.030	0.0063	0.038
	4st	0.0221	0.202	0.0105	0.096	0.0213	0.219	0.0118	0.121	0.0096	0.073	0.0038	0.029
	5st	0.0117	0.145	0.0289	0.361	0.0213	0.265	0.0136	0.170	0.0040	0.040	.....	.....
	6st	0.0160	0.392	0.0092	0.224	.....	.....	.....	.....	0.0032	0.041	0.0072	0.092
	7st	.....	.....	.....	.....	.....	.....	.....	.....	0.0016	0.025	0.0081	0.128
	Top	0.0276	0.214	0.0122	0.094	0.0085	.....	.....	.....	.....	.....	0.0064	0.042
	7L	.....	.....	.....	.....	.....	.....	.....	.....	0.0048	0.029	0.0024	0.014
	6L	0.0243	0.144	0.0092	0.054	0.0064	.....	.....	.....	0.0008	0.005	0.0029	0.016
	5L	0.0291	0.142	0.0060	0.029	0.0064	0.032	.....	.....	0.0024	.....	.....	.....
	4L	0.0416	0.217	0.0064	0.033	0.0235	0.131	0.0073	0.036	0.0048	0.019	.....	.....
	3L	0.0213	0.128	.....	.....	0.0353	0.172	0.0085	0.047	.....	.....	.....	.....

TABLE 5

DISTRIBUTION OF AMIDE NITROGEN IN NORMAL AND RINGED CANES

Fraction	Cane segment	June 23				July 16				August 9			
		Controls		Ringed		Controls		Ringed		Controls		Ringed	
		Gm.	Percentage residual dry weight	Gm.	Percentage control residual dry weight	Gm.	Percentage residual dry weight	Gm.	Percentage control residual dry weight	Gm.	Percentage residual dry weight	Gm.	Percentage control residual dry weight
Amide nitrogen	1st	0.0028	0.012	0.0006	0.003	0.0021	0.009	0.0044	0.019	0.0008	0.003	0.0027	0.012
	2st	0.0020	0.012	0.0013	0.008	0.0123	0.071	0.0061	0.009	0.0008	0.004	0.0030	0.017
	3st	0.0042	0.032	0.0021	0.015	0.0011	0.001	0.0032	0.022	0.0004	0.002	0.0021	0.013
	4st	0.0051	0.047	0.0031	0.028	0.0005	0.001	.....	.....	0.0000	0.000	0.0008	0.006
	5st	0.0030	0.037	0.0009	0.011	0.0021	0.026	0.0005	0.006	0.0014	0.014	0.0008	0.008
	6st	0.0016	0.049	0.0000	0.000	.....	.....	.....	.....	0.0005	0.006	0.0020	0.026
	7st	.....	.....	.....	.....	.....	.....	.....	.....	0.0000	0.000	0.0019	0.030
	Top	0.0024	0.019	0.0011	0.008	0.0021	.....	0.0017	.....	0.0008	0.005	0.0024	0.016
	7L	.....	.....	.....	.....	.....	.....	0.0000	0.0000	0.0005	0.003	0.0020	0.012
	6L	0.0012	0.007	0.0004	0.003	0.0005	.....	0.0000	0.0000	0.0004	0.002	0.0015	0.009
	5L	0.0020	0.010	0.0005	0.002	.....	.....	0.0011	0.006	0.0004	.....	0.0024	.....
	4L	0.0024	0.013	0.0018	0.009	0.0011	0.006	0.0027	0.015	0.0000	0.000	.....	.....
	3L	.....	.....	0.0010	0.006	0.0016	0.008	.....	.....	.....	.....	.....	.....

early life of the canes, if for no other reason than that they were no longer so abundantly available to the plant later in the season. The plants were grown in uniform Palouse silt loam from which nitrates are not uniformly supplied; there is an abundant supply of nitrates early in the spring, but this is soon reduced to a low but not deficient quantity.

The effect of ringing on the rise of inorganic solutes in the xylem has been discussed by CLEMENTS and ENGARD (2). They demonstrated that the ring as a break in the continuity of the phloem does not affect the rise of inorganic solutes, but that the ring, as it affects the movement of water, influences the upward movement of salts in the xylem. This effect is produced by prevention of the development of new xylem at that point, which, as growth above continues, becomes a constriction in the cross-sectional area of the conducting channel; also the ringing process causes incapacitation of a portion of the already existing xylem by destruction of cells and clogging of vessels and tracheids upon exposure to air, thus further limiting the capacity of the xylem to conduct water with its dissolved salts. These effects are partly responsible for the erratic figures for nitrate concentration, especially in the first three segments of the ringed canes. Ringing does not prevent the upward movement of nitrates in the xylem, because nitrates are supplied to and utilized by the plant above the rings throughout the period of treatment. The narrow band of xylem which was present when the plants were ringed in May was rather seriously affected by the double ringing. The partial incapacitation of the xylem is shown in the figures for the concentrations of nitrates in the ringed canes of the June collection (ringed May 15). There is an apparent accumulation of nitrates below the rings, a loss between, and a slight loss above. The accumulation appears to be a partial damming of the transpiration stream by the inability of the wounded xylem to conduct the stream. Ringing later in the season (in June and July) is less detrimental to the plant, for, as the data indicate, the figures for nitrate concentration in the first three segments are more nearly normal.

There are other factors which are probably influential in determining the amounts of nitrate in the xylem, as well as in the phloem, in the various parts of the plant. One of these is differential absorp-

tion from the xylem, determined by the rates of reduction and amino acid formation at various places along the stem. Differential growth from one internode to another in part would determine this, and such growth is characteristic of the Cuthbert raspberry. The absorption of nitrates from the xylem would in part depend upon the relative amounts of other substances (especially the carbohydrates and amino acids) present in the various regions of the stem.

AMMONIUM NITROGEN.—The concentrations of this fraction throughout the plant are lower than, but parallel to, the alpha amino acids, being highest in the subterminal segment (tables 1, 3), with the tops and leaves very low. In all collections there is an accumulation between the rings, further strengthening the parallelism with alpha amino acids. There is an accumulation of ammonium nitrogen below the rings of the plants of the June collection, and a loss below the rings of the plants collected in August. Deaminization of some of the amino acids produced by proteolysis between and below the rings of the June collection accounts for the accumulation of ammonium nitrogen, which apparently does not rapidly escape to the xylem. In the upper regions of the treated plants of the August collection the concentrations of ammonium nitrogen are about normal.

NITROGEN GRADIENTS

MASKELL and MASON (**9, 10**) postulated a relationship between longitudinal movement and concentration gradient. Nitrogen, they found, accumulated above a ring against a strong negative[5] gradient of organic crystalloid nitrogen. They postulated and partly confirmed that this was owing to a small fraction, "residual" nitrogen, which travels downward with a slight positive gradient. The amino acids, which were abundant in the sieve tube layer of the bark of the cotton plant, are thought not to be a translocational form because of the steep negative concentration gradient. The gradient of organic crystalloid nitrogen was positive in the leaves; that is, the concentration was greater in the young than in the old leaves. Total nitrogen was higher in leaves than in bark, but the concentration of organic crystalloid nitrogen was the reverse. Amino acids, plus

[5] Negative is used as opposed to the positive or carbohydrate gradient of higher concentrations in the top, lower concentrations downward.

residual nitrogen, were higher in the leaves than in the bark. In their gradient studies MASKELL and MASON divided the plants into an upper region, in the center of the foliage region, and a basal, non-foliage lower region. Gradients were determined between the two regions and represent the excess of concentration in the upper region over that of the lower region. A negative sign was used to indicate gradients in the opposite direction.

As previously stated, the raspberry plants were separated into 15 cm. segments from bottom to top. The last is a part segment which includes the stem tip and the small unfolding leaves (top). Table 6 presents the total gradient and gradient per centimeter of distance in the normal and ringed stems respectively, and the gradient from the last stem segment (6st or 7st) into the tops for the six nitrogen fractions. The concentrations of total organic nitrogen descend from the highest concentration in the highest stem segment (tops) to lower concentrations in lower stem segments; the gradient is positive downward. The concentrations of the soluble organic nitrogen fractions descend from the highest concentration in the subterminal segment to lower concentrations in the top, the leaves, and lower stem segments; the gradient is positive upward, laterally, and downward respectively.

The leaves and tops are much higher in total nitrogen than any of the above-ground parts. The gradients are steep in the May collection, become less steep in June, and still less by August when metabolism has generally decreased. The gradient per centimeter of stem drops from 0.075 in May to 0.025 in June and 0.004 in August.

The gradients of the total soluble nitrogenous fractions, like those of the total organic nitrogen, exhibit the decrease in gradient per centimeter of stem from May to August. Each fraction contributes to this decrease in the total gradient, nitrates exhibiting the greatest decrease in gradient per centimeter of stem from May to August.

Table 6 presents the effect of ringing on gradients in comparison with gradients in the normal stems. The same general decreases in gradients are shown by these figures as were shown by those of the normal plants, but there are slight modifications. The gradients per centimeter of stem of the soluble organic fractions are greater than

those of the corresponding controls. The gradients in the control and treated plants are less than the beginning control (May) gradients. The gradients of nitrate and ammonium nitrogen are generally less than those of the corresponding controls. There is no effect of

TABLE 6

CONCENTRATION GRADIENTS IN NORMAL AND RINGED CANES

DATE	NITROGEN FRACTION	NORMAL			RINGED		
		SUBTERMINAL TO BASAL		SUBTERMINAL TO TOP	SUBTERMINAL TO 3ST		SUBTERMINAL TO TOP
		TOTAL GRADIENT	GRADIENT PER CM.		TOTAL GRADIENT	GRADIENT PER CM.	
May 15	Total organic	+2.250	+0.075	+0.610*			
	Total soluble organic	+0.862	+0.029	+1.265†			
	Alpha amino	+0.351	+0.012	+0.448			
	Ammonium	+0.142	+0.005	+0.383			
	Nitrate	+0.410	+0.013	+0.374			
	Diamino	+0.010	+0.000	+0.010			
June 23	Total organic	+1.908	+0.012	+1.785	+1.240	+0.028	+0.740
	Total soluble organic	+1.072	+0.014	+0.795	+0.680	+0.015	+0.596
	Alpha amino	+0.099	+0.001	+0.025	+0.349	+0.008	+0.280
	Ammonium	+0.350	+0.005	+0.294	+0.114	+0.003	+0.126
	Nitrate	+0.357	+0.005	+0.178	+0.156	+0.003	+0.130
	Diamino	+0.174	+0.002	+0.097	+0.135	+0.003	+0.157
August 9	Total organic	+0.355	+0.004	+2.416	+1.036	+0.017	+1.010
	Total soluble organic	+0.420	+0.005	+0.110	+0.914	+0.015	+0.772
	Alpha amino	+0.058	+0.001	+0.012	.....	.....	.....
	Ammonium	+0.055	+0.001	.....	+0.019	+0.000	+0.012
	Nitrate	+0.019	+0.000	.....	+0.090	+0.002	+0.086
	Diamino	+0.177	+0.002	+0.128	+0.228	+0.004	+0.203

* Plus (+) = concentration highest at top.

† Plus (+) = concentration lower in top.

ringing on the direction of the gradients between the terminal and subterminal segments, but there is a general reduction in steepness.

The gradients reveal little more than differences in concentrations in the various parts of the plants; changes in gradients reveal only changes in relative concentrations. The decreasing gradient per centimeter of stem indicates a tendency of the fractions to become more nearly equal in all parts of the maturing plant. Ringing modifies the

relative concentrations of nitrogenous substances, probably through modifications in the growth of the upper region of the plant.

The steepness of the gradients of nitrogen fractions in the May plants as compared with those of the June plants would appear to indicate in part a downward movement of nitrogen from the upper regions. The rings, however, would cause an accumulation of a

TABLE 7

NITROGENOUS SUBSTANCES IN GRAMS IN NORMAL PLANTS OF MAY AND NORMAL AND RINGED PLANTS OF JUNE*

	TOTAL ORGANIC NITROGEN	TOTAL SOLUBLE ORGANIC NITROGEN	ALPHA AMINO NITROGEN	DIAMINO NITROGEN	NITRATE NITROGEN	AMMONIUM NITROGEN
May						
Stem	2.149	0.888	0.248	0.059	0.251	0.313
Leaves	2.961	0.524	0.104	0.092	0.216	0.083
Total	5.110	1.412	0.352	0.151	0.467	0.396
June						
Normal						
Stem	1.323	0.423	0.101	0.057	0.112	0.089
Leaves	2.448	0.261	0.060	0.045	0.117	0.017
Total	3.771	0.684	0.161	0.102	0.229	0.106
Ringed						
Stem	1.513	0.435	0.138	0.050	0.081	0.103
Leaves	1.842	0.193	0.069	0.029	0.022	0.015
Total	3.355	0.628	0.207†	0.079	0.103	0.118

* Figures are for samples of 12 plants per sample.

† Increase in this fraction produced by accumulation of alpha amino acids between and below the rings.

downward moving organic form of nitrogen in the bark, and the gradient per centimeter of stem above the rings would be reduced greatly. Table 6 shows that the gradient is actually steepened for all fractions except ammonium and nitrate nitrogen.

That there is a considerable loss of nitrogen from the above-ground portion of the plant is shown in table 7, which gives the grams of nitrogenous fractions found in the whole plant (figures obtained by adding the grams per sample figures in tables 1 to 4, each sample representing twelve plants). From May 15 to June 23 there is a great decrease in the amount of all nitrogenous fractions.

The rings had little effect on the amount lost from the above-ground portions of the plant. The grams of nitrogen in the portion of the plant above the rings are compared with the amount of nitrogen in the corresponding portion of the normal plant in table 8. If there were a downward movement of nitrogenous substances in the bark—and substantial movement would be necessary to account for the

TABLE 8

NITROGENOUS SUBSTANCES IN GRAMS IN NORMAL PLANTS OF MAY AND NORMAL AND RINGED PLANTS OF JUNE IN PORTION OF PLANT ABOVE POSITION OF UPPER RING

	Total organic nitrogen	Total soluble organic nitrogen	Alpha amino nitrogen	Diamino nitrogen	Nitrate nitrogen	Ammonium nitrogen
May 15						
Stem*	0.839	0.258	0.078	0.018	0.090	0.074
Leaves	2.961	0.524	0.524	0.092	0.216	0.083
Total	3.800	0.782	0.602	0.110	0.306	0.157
June 23						
Normal						
Stem	1.034	0.326	0.076	0.047	0.091	0.071
Leaves	2.448	0.261	0.060	0.046	0.117	0.017
Total	3.482	0.587	0.136	0.093	0.208	0.088
Ringed						
Stem	1.150	0.274	0.079	0.038	0.070	0.046
Leaves	1.842	0.193	0.069	0.030	0.022	0.015
Total	2.992	0.467	0.148	0.068	0.092	0.061

* These figures represent only nitrogenous fraction in one segment plus stem tip of 10 cm. length or less, whereas figures for stem of June plants represent quantities in four stem segments plus tip.

loss of nitrogen from May 15 to June 23—there would be a definite accumulation above the rings. This did not occur, as table 8 shows, for the amounts of nitrogen above the rings are even slightly lower than those of the corresponding portion (above the position of the rings) of the normal plants harvested at the same time. Table 8 shows that the one segment of the normal May plant, the only segment remaining above the position of the upper ring on corresponding treated plants, contains (with the stem tip) more nitrogen than the four segments and stem tip above the rings of the treated plants of June or of the corresponding portion of the June control plants.

The bulk of the nitrogen in the May plants is in the first two segments (table 1); some of this may be redistributed to the upper segments as the cane increases in length and amount of foliage, and some may be lost either to the roots or to the environment. Instead of accumulation, therefore, there is continuous loss of nitrogen from above the rings, and downward movement in the bark is not involved in the loss.

The loss of nitrogen from May 15 to June 23 is great, of the order of 16–22 per cent, and occurs during the period of most rapid growth.[6] The concentrations in the plants remain at about the same level from May to August, perhaps decreasing a little. The data for all segments of the July and August plants are not complete, so an exact comparison in grams for the whole plant cannot be made, but comparison of the amounts and concentrations in the segments for which there are data bears out this statement. The nitrogen involved in this early spring loss either moves out of the stem to the roots, or is lost to the environment directly from the plant. As pointed out from the data in table 8, there is no accumulation of nitrogenous substances above the rings during the five weeks after ringing, which indicates that there is no downward movement in the bark. It is hardly conceivable that such a great loss of nitrogen from the above-ground portion of the plant took place by ordinary diffusion in the xylem, especially under the conditions of very high transpiration rate which exists during the summer in eastern Washington. A loss of nitrogen of a similar magnitude has been recorded by DONEEN (**5**) for varieties of wheat grown in this locality, and the loss was assumed to have resulted from translocation to the roots. WILFARTH, RÖMER, and WIMMER (**22**) reported a loss of nitrogen from several plants. They thought the nitrogen was translocated to the roots, but no analyses were made on the roots. Other investigators have reported losses of nitrogen for which there was no definite accounting. There are several possible ways in which a loss of nitrogen could occur without transport to the roots. One of these is a loss directly to the atmosphere as gaseous nitrogen; the other is washing out by rain.

[6] The plants increased from approximately 50 to 100 cm. in length, and in dry weight from 161 to 202 gm. in the five weeks' interim.

Discussion

The gradients reported here on the basis of residual dry weight do not indicate the direction of movement or even the existence of a dominant translocational form of nitrogen. The concentration gradients represent little more than gradients of the ratio of living, protoplasm-containing cells to non-living, protoplasm-lacking cells, which is greater in the top of the stem than in any other part. Protoplasm is densest in the meristematic regions, and the highest concentrations of total organic nitrogen are found in the top segment of stem (which includes the stem tip and the young, unfolding leaves) and in the leaves. The protein constituent of the total organic nitrogen is greatest in the top segment and in the youngest leaves. The soluble forms of organic nitrogen are most highly concentrated in the subterminal segment. Amino acids are partly responsible for the soluble nitrogen gradients, their concentration being also very low in stem top and leaves. The protein gradient is the reverse. It appears, then, that the stem tip and the leaves are the greatest protein synthesizing organs of the plant, for amino acids are utilized very rapidly in these regions. The concentration of protoplasm is greatest in these organs.

That the positive gradients do not indicate a downward movement of nitrogen in the bark is revealed by the lack of an accumulation above the rings and by the failure of the gradient to reverse or even to become level. That an upward movement of an organic nitrogenous form in the bark is not important is indicated partly by the lack of a significant accumulation below the rings but more convincingly by the presence above the rings of an amount of nitrogen which is nearly normal. If nitrogen were supplied to the upper regions of the plant chiefly through the bark, upward movement beyond the rings would be prevented and the nitrogen content of the upper region after five weeks would be greatly below normal. Protein synthesis continues above the rings throughout the period of treatment. The nitrate used in this synthesis must have been supplied by the xylem. Apparently nitrate is the important translocational form of nitrogen in the raspberry plant. Nitrates are abundant early in the spring, and rapid utilization throughout the whole

plant results in considerable amounts of organic nitrogenous compounds. The nitrates move freely in the xylem and are available for amino acid synthesis at all points in the plant.

Differential synthesis of protein and utilization of amino acids account for the gradients between stem and leaves and between stem tip and subterminal segment. The same factors are responsible for the descending gradients in the stem, for the amount of amino acid and protein synthesis is proportional to the varying amounts of protoplasm produced throughout the length of the stem. The phloem is probably the most important stem tissue involved in amino acid synthesis. The function of cortex in this process can be ruled out owing to the production of phellem by a pericyclic phellogen early in the life of the plant. Microchemical tests with diphenylamine reveal nitrate in the phloem as well as in the xylem, not only in the raspberry but in other plants as well. Investigations by Cooil (3) in progress in this laboratory reveal nitrate in the phloem exudate of cucurbits. Moose (14) has demonstrated in several plants the presence of nitrate and other minerals in the phloem exudate, as well as in the "parenchyma sap" of the leaves. The presence of these inorganic constituents in the phloem he thought was "presumptive evidence that inorganic as well as organic materials are transported in the phloem." It has been demonstrated many times that small quantities of salts move in the phloem, but this movement is of a secondary nature, for, as Clements (1) has pointed out, the upward movement of soil solutes takes place chiefly in the xylem, downward movement of salts can occur in the phloem, and the bark may obtain salts directly from the xylem as well as from the leaves. The major, rapid movement of salts is in the xylem (1, 2). In addition to these minor salt movements in the phloem, it is well established that metabolically active protoplasm absorbs salts against a strong negative gradient; that is, with an increasing chemical potential. This process is characteristic of absorption of salts by roots; the absorption of nitrates and other inorganic substances from the relatively passive xylem by a metabolically active phloem is a homologous process. Thus lateral absorption from the xylem accounts for much of the inorganic solutes in phloem tissue. In most instances Moose (14) observed greater quantities of salts

in the leaf parenchyma extract than in the phloem exudate, which itself included phloem parenchyma juice.

The data presented here for the raspberry plant bear out that part of MASKELL and MASON'S assumption which states that the bulk of the mineral nitrogen absorbed by the roots is carried in the transpiration stream. Elaboration of the nitrates to amino acids and protein is not restricted to leaves only, however, but occurs in all living cells of the plant where the proper conditions for nitrate reduction exist. The data for the raspberry do not indicate elaboration in the leaves and exportation through the sieve tubes back to the roots—the second part of MASKELL and MASON'S assumption. On the contrary, the data indicate that the amount of synthesis of amino acid and protein is dependent upon the amount and activity of protoplasm, all other conditions for nitrate reduction being favorable, and is not a function of special organs, although in some plants, especially perennials (18), synthesis of amino acids in the roots appears to exceed utilization in protein formation. In such an instance, upward movement of amino acid appears to take place in the phloem.

Although no definite longitudinal polar movement of organic nitrogen is indicated in the bark of the raspberry, the possibility of local movements of organic nitrogenous substances from regions of higher to lower concentrations still exists. Differential utilization of amino acids in various localized regions may result in a movement of this form of nitrogen. Possibly an example of this is a movement of soluble organic nitrogen into the top from the subterminal segment. This movement could be responsible for the gradient of organic nitrogen existing between these regions, but it must be emphasized that the gradient does not indicate that such a movement takes place and may indicate only that differential utilization has occurred. The important mobile form may well be nitrate, which is supplied to both regions. Examples of definite movement of a substance are found, however, in the considerable movements of carbohydrates into ripening fruits, the movement of sugar into the cotton boll (11), etc.

Certainly there is no well founded reason to assume that the

seat of organic nitrogen synthesis is only in the leaves. Nitrogen reduction takes place wherever there is sufficient carbohydrate, reducase, a proper pH, a proper temperature, and essential elements. Many workers, notably ECKERSON (**6**), THOMAS (**18**), NIGHTINGALE *et al.* (**16, 17**), TIEDJENS (**19, 20, 21**), and LEONARD (**8**), have shown that synthesis may take place to great extent in organs other than the leaves.

Summary

1. Data are presented concerning the distribution and translocation of total organic, total soluble organic, alpha amino, diamino, amide, ammonium, and nitrate nitrogen in the vegetative canes of the Cuthbert raspberry. Double rings were used to check the movement of nitrogenous substances, one ring 15 cm. and the other 30 cm. above the ground. The plants were cut for analysis into 15 cm. lengths. Leaves were analyzed separately from stem segments.

2. There was a steady increase in the concentration of total organic nitrogen from the basal segment to the top. Total soluble organic nitrogen and alpha amino acids attained a maximum concentration in the subterminal segment. Total organic nitrogen was higher in the leaves than in the stem, but the total soluble nitrogen and amino acids were about 50 per cent lower in the leaves than in the stems. The concentration of these substances was about 50 per cent lower in the terminal than in the subterminal stem segment. Protein was therefore most abundant in the stem top and leaves.

3. Nitrogenous compounds did not accumulate in the upper regions of the ringed plants. There was not a significant accumulation below the rings. Increase in the concentration of soluble nitrogen in the necrotic segment between the rings was owing to production of amino acids by proteolysis.

4. All nitrogen gradients were positive, and are thought to be little more than manifestations of the ratio of the amount of living, protoplasm-containing cells to non-living, protoplasm-lacking cells in the various parts of the plant. Thus the concentration of total organic nitrogen is greatest in the top segment and leaves, where there is least differentiation and thickening of cells and much meri-

stematic tissue. The gradient of soluble nitrogen from subterminal to top segment (positive upward), and from stem to leaves (positive laterally), seems to be a manifestation of the differential utilization of the soluble forms, with the greater amount of protein synthesis taking place in the top and leaves, rather than an indication of movement of nitrogen. The gradients in the stem (positive downward) are the result of synthesis or accumulation of nitrogenous substances in the living cells.

5. There appears to be considerable loss of nitrogen from the above-ground portion of the plant from May 15 to June 23. Since there is no accumulation above the rings, the 16–22 per cent loss cannot be explained by movement to the roots in the bark. It is hardly conceivable that it was translocated to the roots in the xylem, in which the transpiration stream is under a great negative tension owing to a high rate of transpiration. It is suggested that the loss is in the form of a gas, or occurs through leaching by rain.

6. It is concluded that there is no definite longitudinal translocation of organic nitrogen either upward or downward in the bark. Nitrate is the important translocational form of nitrogen in the Cuthbert raspberry. It rises freely in the xylem, and is available for reduction and elaboration to amino acids and protein not only in the leaves but in all living cells of the plant where conditions favorable to reduction exist. The amount of nitrate reduction and elaboration is proportional to the amount of protoplasm in the various parts of the plant; thus the greatest amount of protein is found in the stem top and leaves. Reduction and synthesis take place also in the stem and in proportion to the concentration of protoplasm per unit of weight; hence the existence of a positive concentration gradient.

7. In the stem the phloem is a highly metabolic tissue, and absorbs nitrates and other inorganic salts from the relatively passive xylem. The presence of inorganic solutes in the phloem is the result of an absorption process homologous with the absorption of salts by roots, and is not a manifestation of primary solute translocation.

The writer wishes to express his gratitude to Dr. H. F. CLEMENTS of the University of Hawaii, who suggested this problem in conjunc-

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ANATOMY OF THE AERIAL AXES OF EQUISETUM KANSANUM

ISABEL M. P. BROWNE

(WITH ONE FIGURE)

Introduction

In 1912 Professor J. H. SCHAFFNER described as *E. kansanum* a new species of *Equisetum* that had until then been confused with *E. laevigatum* A. Br., from which it differed chiefly in that its aerial stems were annual and its cones devoid of a specialized, rigid apical point. The specific diagnosis ran: "Aerial stems usually 1–2½ ft. high, annual, very smooth, 15–30 grooved,[1] usually without simple branches, unless broken off; color mostly light green; surface of the ridges and grooves with cross and diagonal bands; sheaths long, dilated above and usually constricted at the base, green with a narrow black band at the top; teeth deciduous; cones ovate or oblong-ovate, without a point, the apex obtuse or merely acute" (4, p. 21).

SCHAFFNER most kindly sent me from Ohio material of his new species, duly fixed and preserved for anatomical investigation. He was also good enough to discuss with me by correspondence the nature of the new species. For these privileges I wish to thank him sincerely.

The following short account of the anatomy of the aerial stems of *Equisetum kansanum* Schaffner is based on microtome sections of nine axes.[2] Serial transverse sections were made of two large, of one medium sized, and of two small stems. Of one of the large stems the series extended from the upper end of a node downward through that node, through a complete internode, through a second node, and through part of the internode below the latter. In the other cases the portions included a complete node with portions of the inter-

[1] In the largest of my specimens (fig. 1), thirty-seven grooves and bundles were observed.

[2] Some of the smaller axes sent me may have been branches, but this is unlikely, as branching of aerial stems is rare. Moreover, all but one of these small axes bore cones, and there seems to be no record of fertile branches.

nodes above and below. The three small branches were fertile and in each case the node sectioned was the uppermost vegetative one. Serial longitudinal sections were made through four axes. In all of them the part sectioned included a complete node with portions of the internodes above and below. One of the stems was very large, one was of medium size, and two were very small. One of the latter was fertile and in it the node sectioned was the uppermost vegetative one.

Observations

Large stems

Stem *A*, the largest of those cut transversely, had thirty-seven bundles and ribs, and at its widest was 6 mm. in diameter. At the level of the diaphragm it narrowed, the leaf sheath being constricted at the base to a diameter of less than 5 mm. The other large axis cut transversely, stem *B*, had thirty-two ribs and bundles below the lower of the two nodes. When the ring of nodal xylem broke up, only twenty-nine bundles were reconstituted, each lying opposite a rib. This was also the number of leaves and leaf traces at the next node; but, rather surprisingly, on the breaking up of this (upper) nodal ring of xylem thirty-two bundles were once more reconstituted, each lying opposite a rib. A few of these were unusually small and it is possible that some of them died out a little higher up, although the sections did not extend far enough to show whether this was so. At its widest, stem *B* attained a diameter of about 4.5 mm., while at the level of the diaphragm it was but 4 mm. wide. In the large stem sectioned longitudinally, stem *C*, the axis at its widest was about 4.25 mm., while at the level of the diaphragm it was only about 2.75 mm. wide. This stem was very young, and above the diaphragm the tissues were immature for a distance of about 6 mm. At this level the axis was in a very youthful phase, most of its cells being small and in process of rapid division.

As in *Equisetum laevigatum*, the furrowing of the stem is not very conspicuous, the grooves being wide and shallow. The ribs are rounded and externally convex, without indication of the biangular form with slight median concavity found in *E. laevigatum* (**2**, pl. xxxii, fig. 3). *E. kansanum* is one of the Equiseta cryptopora, species

with stomata sunk beneath the surface of the epidermis. The stomata are disposed in two uniseriate rows in each groove, each row lying on one side of the latter, about halfway between its middle and the middle of the neighboring rib. The stomata of a row are separated from one another by a single short cell. Each stoma abuts on a schizogenously formed air space.

In the mature regions of the internode, two to three rows of long, narrow, pointed fibers lie immediately under the epidermis. Under the ribs the fibers are more numerous, and so disposed that in transverse section they form a triangular tooth, projecting inward toward the bundle. In a radial direction this tooth may contain from six to twelve fibers. Shorter teeth, composed of similar fibers, sometimes project inward from the middle of grooves, although in this position but few additional fibers are developed and the teeth rarely attain more than half the radial length of those under the ribs, while they are often completely absent. The extent of development of the fibrous teeth under the ribs and grooves varies very much, not only in different stems and different parts of the same stem, but even in adjacent ribs and grooves of the same section.

Since in *Equisetum*, differentiation of the tissues proceeds in each articulation from above downward, the cells just below the node are often in a more mature condition than are those lower down in the internode; and at this level the tip of a projecting tooth of fibers under a rib may be connected with the bundle on the same radius by a single (rarely locally double) chainlike row of somewhat radially elongated cells with slightly thickened walls. It seems to be rare to find this condition in neighboring bundles, and in my specimens at least, such a row of cells had a very slight vertical extension—not more than 70 μ. A similar development occurs, although rarely, lower down in the internode. One of MILDE's figures of *E. trachyodon* A. Br. shows a very similar conformation of the cells (**2**, pl. xxxiii, fig. 14).

The assimilating tissue (MILDE's *grüne Parenchymschicht*) lies immediately under the subepidermal layers of fibers. It consists of two to four (usually three) rows of cells with thin undulating walls. These cells, which are differentiated at an early stage, have dense contents and form a loose spongy tissue. Those of the innermost row

are more or less isodiametrical and close to one another. Those of the outer two rows are radially elongated and between them are developed schizogenous air spaces wider than the cells. In a longitudinal section of the stem these layers have a very characteristic, ladder-like appearance; although when, as is frequently the case, the middle cell of the three joins on to two cells (one belonging to its own and one to a neighboring row), the bars of the ladder seem to fork. In longitudinal section these bars always seem to slope obliquely upward, presumably an effect of growth. As seen in a transverse section of the axis, the external outline of the assimilating tissue is determined by the absence or presence and degree of development of the fibrous toothlike projections. Opposite the ribs of the stem it extends in most cases inward to within a layer or two of the outer endodermis of the bundle; opposite the grooves it is generally bounded internally by the outer margins of the vallecular canals. Even before the latter are formed, their limits are distinctly visible. The cells that are later destroyed by the formation of the vallecular canals are much larger than those of the assimilating tissue, and begin to lose their contents very early. In a transverse section of the stem they form oval tracts of thin-walled, collapsible looking tissue, of which the middle cell is the largest and also the first to be destroyed. In a typically internodal section the vallecular canals are from two to four times as wide transversely as radially; but as the node is approached and the bundles increase in size, the canals become narrower.

In the internode, outer and inner ground tissues are completely separated by an endodermis or rigid sheath of small cells, the radial walls of which do not show the typical endodermal markings. This external endodermis runs round the periphery of each bundle and follows the contour of the latter for some distance, usually until it reaches about the depth of the middle of the carinal canal. It then bends more or less at right angles and runs to the next bundle, and bending again outward, follows the outer part of the contour of this bundle. The outer endodermis thus surrounds the peripheral part of each bundle in succession. There is also an inner endodermis running along the inner edges of the bundles; this too is continued between

the bundles, either running conformably to the circle in which the latter are arranged or curving a little outward between them. The cells abutting on the sides of the bundles and lying between the outer and inner endodermes also develop as part of the sheath. In this region and at the periphery of the bundles the outer endodermis often becomes two cells thick, the cells of the outer row being rather the larger. On looking at a transverse section of the stem, all three forms of endodermis—outer, inner, and special—seem to be developed.

As early as 1867, PFITZER gave a clear description of the inner and outer endodermes that he had found in the stems of *E. hyemale* L., *E. trachyodon* A.Br., *E. ramosissimum* Desf., and *E. variegatum* Schleicher (**3**, pp. 310–312). As can be seen by comparing his figure 8 of pl. xviii showing a transverse section of two bundles from the internode of *E. variegatum* with my figure of two bundles of the internode of *E. kansanum*, it would be necessary only for about two cells on each side of the bundle, near its inner edge, to be differentiated as cells of the sheath to convert the type of sheath figured by MILDE into the kind found in *E. kansanum*. In the latter species the endodermal sheath system seems to be much more developed than in the species with inner and outer endodermes, described by PFITZER. He stated that in the region between the bundles, inner and outer sheaths were usually separated by one or more (rarely by two) layers of cells, but that in a few cases they were in contact (**3**, p. 311). In *E. kansanum* inner and outer endodermes are in contact throughout their course; and a third—or even locally a fourth series—of cells may be added to the sheath, either internally or externally, or on one or on both sides and for part or for the whole of the distance between two bundles.

In a longitudinal section it can be seen that when mature, the internodal cells of the sheath are vertically elongated, although very narrow radially. Transverse sections show that between the bundles the cells of the sheath are somewhat elongated tangentially, although not nearly so much so as vertically. Even vertically they are not so long as the tracheids and their ends are blunt, the transverse

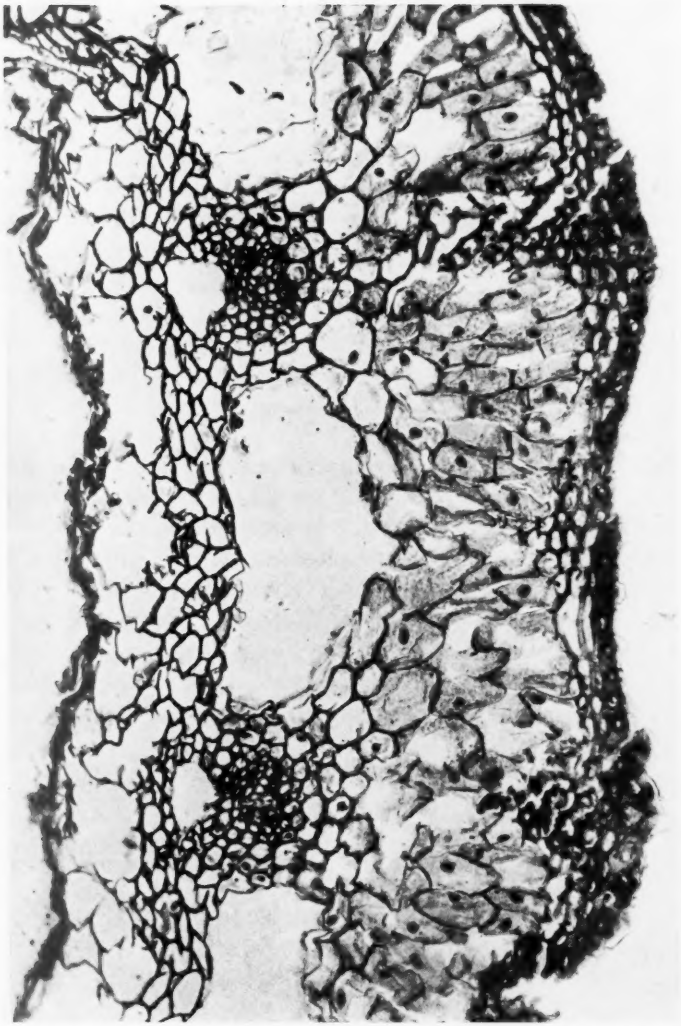

FIG. 1.—Transverse section from large stem of *E. kansanum*. Note inwardly projecting teeth of fibers of unequal length under ribs and grooves, and row of cells, somewhat elongated radially and with slightly thickened walls, connecting tip of one tooth with bundle on same radius. Section traverses two stomatal apparatus (which lie in shallow furrows on either side of middle of groove) at different levels. On right the section passes through part of a schizogenous air space below a stoma. Note well developed multiseriate endodermis and, abutting on central cavity, disorganized remains of cells destroyed by formation of that cavity. These remains consist chiefly of walls of collapsed cells and probably correspond to tissue erroneously interpreted and figured by MILDE as a narrow band of delicate or gauzelike cells delimiting the central cavity.

walls being commonly somewhat oblique. After staining, these walls show numerous small, irregularly placed light spots, probably pores.[3]

Except in the neighborhood of the nodes, the bundles are oval in outline, with the longer axis directed radially, and far apart, the distance between them being from two to four times as great as their own width, or sometimes even more. In mature regions of the internode the carinal canal is often relatively large and may occupy half, or more, of the space within the sheath of the bundle. There is great variety in the shape and size of the carinal canals. In two adjacent bundles of the same section one carinal canal may be half the size of its neighbor, and round or oval; if oval, the longer axis may be radial or transverse. The metaxylem elements are usually arranged in two nearly radial rows, each row abutting on the lateral part of the sheath surrounding the bundle and curving slightly inward, conformably to the shape of the sheath. Typically the rows of metaxylem are but one tracheid in width; frequently there are two tracheids, side by side, at the inner end of a row; and not infrequently one and occasionally both rows are replaced by small groups of tracheids. But whether the tracheids are in a row or a group, the narrower end of the metaxylem is toward the periphery of the bundle. Here there is a single narrow tracheid. Each row or group consists of from two to seven elements, four to six being the usual number. Sections through the regions where the metaxylem is not yet completely developed show that the direction of differentiation is centrifugal, as it is in all the species (*E. arvense*, *E. palustre*, *E. limosum*, *E. maximum*, *E. hyemale*, *E. sylvaticum*, *E. debile*, and *E. variegatum*) in which this point has been elucidated (**1**, pp. 458–459) except *E. giganteum*. It is remarkable that this centrifugal development seems to be less subject to irregularities in *E. kansanum* than in the other species studied, and this although the diameter of the metaxylem tracheids decreases steadily and markedly in size from within outward.[4] It would seem that the more peripherally

[3] Similar but less well marked pores were observed on the transverse walls of some of the larger cortical cells abutting on the endodermal sheath. PFITZER (**3**, p. 333) appears to have observed somewhat similar small pores on the vertical walls of the cells of the sheath and of the parenchymatous tissues at the node.

[4] In a transverse section of the stem it sometimes seemed as though a more deeply seated tracheid were smaller than a more peripherally situated one, but this was due to the plane of the section passing through either of the narrow ends of a tracheid.

situated cells of the bundle divide more frequently and more rapidly than those toward its interior, and are thus considerably smaller than the latter at the time of their lignification.

The lateral groups of metaxylem are differentiated much later than the protoxylem, and the latter has usually been replaced by a carinal canal before the first metaxylem tracheid is differentiated. Occasionally one group of metaxylem develops before the other; and apparently in some cases one group failed to differentiate at all over a short vertical distance (*cf.* **2**, pl. xxxiii, fig. 15, *E. trachyodon*). In mature parts of the internode, both the phloem and the parenchyma lying between the lateral bands of metaxylem may become lignified. In mature regions of the internode the central cavity occupies four-fifths or an even higher proportion of the axis. It forms very early, while the cells which will be replaced by vallecular canals are still indistinguishable from their neighbors. Destruction of the centrally seated parenchyma of the stele and of the protoxylem begins at the same level, a little above the diaphragm, while the cells of the ground tissue are still in a very youthful condition. Near the center of the axis a few torn remains of ground tissue persist above the diaphragm. The cavity widens rapidly; and while the epidermal and cortical tissue and that of the vascular bundles (with the exception of the protophloem and protoxylem) are highly immature and the cells composing them are still undergoing division, the whole of the protoxylem and all the ground tissue internal to the bundles—except four to seven rows immediately abutting on the inner endodermis—have perished. As a rule, even in mature stems two to three rows of large thin-walled cells persist inside the inner endodermis, although they too may sometimes disappear for a short distance. The collapsed remains of some of the last cells to be destroyed cling to the edge of those that persist, inside which they form a continuous, narrow band. As the walls are the portions of the cell that resist destruction longest and as they are deposited in layers but slightly separated from one another, their appearance is not unlike that of layers of narrow delicate cells. MILDE has figured and described such layers of approximated, delicate, thin-walled cells bordering on the central cavity in *E. myriochaetum*, *E. hyemale*, *E. laevigatum*,

and *E. trachyodon*.[5] MILDE (**2**, p. 516) calls the cells of these layers delicate, or very or extremely delicate, even gauzelike (*florartig*). It seems unlikely that destruction of the ground tissue would be arrested at a spot where the nature of the cells is such that they would offer very little resistance. Therefore, and because of the similarity of his figures of these layers to the layers occupying the same position in *E. kansanum*, I think it probable that MILDE, working with herbarium material, mistook such torn remains of cells for intact layers of narrow, delicate cells. His figure of *E. trachyodon* seems to be the one which in regard to the tissues between the endodermis and the central cavity comes nearest to *E. kansanum*. Here, as is common in *E. kansanum*, a few layers of large thin-walled cells persist internally to the endodermis.

NODAL STRUCTURE

At the level of the node of stem *A* one, at that of the lower of the two nodes of stem *B* three, and at that of the node of stem *C* two, branches were initiated. None of these branches broke through the leaf sheath and their presence was revealed only by study of the sections. No material difference was observed in the manner of attachment of the vascular supply of these branches to the axial stele from what has been recorded for the genus; and in the following short account of the nodal structure of *E. kansanum* the complications introduced by the presence of these dormant branches, as well as those due to a change in number of the bundles of the successive nodes and internodes of stem *B*, will be left out of account. The irregularities resulting from these causes are confined to a relatively narrow sector of the stele.

As the node is approached from below, the vascular bundles and the tracts of persistent parenchyma connecting them with the fibers under the ribs increase in width. The vallecular canals consequently become narrower, and for a distance below the node their longer axis is radial. Higher up, but still below the level of the diaphragm, the outline of the vallecular canals becomes for a short time circular.

[5] *Cf.* my figure 1 and **2**, pp. 498, 516, 549, 560; and pl. xxvii, fig. 6; pl. xxix, fig. 19; pl. xxx, fig. 23; pl. xxxii, fig. 5; and pl. xxxiii, fig. 14. In the case of *E. trachyodon*, the word used by MILDE for approximated is *genährte*, clearly a misprint for *genäherte*.

This is due to the fact that vertically above the space occupied by the inner parts of the vallecular canals (which through most of the internode reach inward to about half the radial depth of the bundles) nodal tracheids are developed by the enlarging vascular bundles.

Within the bundle the processes at the node resemble in essentials those described for other species of *Equisetum*. That is to say, the tracheids in the lateral groups of metaxylem increase in number and in size; a transverse bar of xylem develops, joining up the two lateral groups, and this gradually thickens until the metaxylem forms an oval mass. The tracheids are of the usual nodal type found in *Equisetum*—short, wide, and reticulately thickened. Meanwhile the carinal canals have disappeared, nodal tracheids occupying their place, except where the protoxylem destined to the trace persists—in a median position at the inner edge of the bundle. Before departure of the protoxylem, a narrow parenchymatous gully forms, running through the middle of the bundle. This gully develops from within outward and passes through the bundle obliquely upward. The protoxylem passes slowly and steeply upward and outward through it, the peripheral part of the nodal xylem being still unbroken when the protoxylem is passing through the innermost part of the gully. Typically all the protoxylem passes out into the trace, and as seen in transverse section of the latter there are about six to eight elements of protoxylem. Once or twice a tracheid or two seemed to be left behind in the axis; then such elements died out almost at once. The protoxylem of successive internodes is therefore completely discontinuous. Some of the nodal tracheids from the sides of the parenchymatous gully, near its outer end, often pass out in the trace; but the xylem of the latter is chiefly composed of protoxylem. Each trace is surrounded by a separate sheath, or endodermis, and this is derived from the external two-layered endodermis of the axis. As the traces pursue a steep outward course through the cortex, the abaxial halves of their sheaths are continuous with the outer layer and the adaxial halves with the inner layer of the two-rowed external endodermis of the stem.

It seems characteristic of the large axes of this species that full development of the nodal xylem is not attained until after the protoxylem of the trace has become completely free from the axial

xylem. During the slow progress of the protoxylem through the parenchymatous gully, the bundles are in contact laterally by their endodermes. The endodermal cells on the sides of the bundles are subsequently replaced by nodal tracheids and the xylem of neighboring bundles becomes confluent. As the nodal xylem is of slight vertical extent and as the bundles do not all develop their nodal xylem at exactly the same level, no single transverse section of the axis shows a complete ring of nodal wood. When the trace is set free from the axial stele, the inner and outer layers of the external endodermis unite round it, so that it is as it were nipped off. Opposite the points of departure of the traces, the nodal xylem becomes thinner radially, and at these points it breaks somewhat higher up, the newly constituted bundles alternating with those of the internode below. When the nodal ring of xylem breaks up, inner and outer endodermes become invaginated and meet round the bundles, so that these when first constituted form transversely elongated oval masses, separated from one another by a single row of endodermal cells. Soon this layer duplicates, and the resulting layers themselves duplicate, so that the xylem of adjacent bundles is for a time separated by four endodermal cells. Parenchymatous cells then appear between the double endodermes; and the bundles, steadily narrowing, come to lie farther and farther apart.

There seems to be some variety as to the level at which protoxylem appears in the internode, but in my specimens it did not do so until after the bundles with their separate endodermes had been reconstituted; it usually reappeared from 200 μ to approximately 220 μ above its disappearance into a trace. Soon after the appearance of the first nodal tracheids, narrowing of the outline of the circle formed by the xylem of the axis begins. This narrowing continues in passing upward, until at or slightly below the level of the diaphragm the diameter of the circle formed by the xylem is at least 1 mm. less than at a definitely internodal level. This is well seen when a longitudinal section passes more or less radially through a bundle; the latter then seems to be terminated just below the diaphragm by an incurved, club-shaped mass of short, wide, reticulate tracheids, these forming part of the ring of nodal tracheids.

The sclerized diaphragm is situated at the level at which, after

the traces have become free from the axial stele, the nodal xylem is dying out in the reconstituted bundles and being replaced by carinal canals, containing as a rule remains of protoxylem. It consists of two layers of cells (although in parts only one of the two superposed cells is definitely sclerized), except at the periphery, where there seems usually to be but one sclerized layer. The cells of the diaphragm are vertically very short but tangentially elongated, so that in a longitudinal section of the stem they seem brick-shaped. As early as 1867 PFITZER (3) noted that at the node the various types of cell became shorter and had thicker walls. A few remains of parenchymatous cells persist just below the diaphragm, and toward the periphery of the central cavity they are rather more numerous and less torn. These cells are also somewhat shortened, but not so much so as the cells of the diaphragm, from which they are sharply differentiated.

In the largest axis studied the diaphragm became gradually more and more depressed, although but slightly so, toward the center of the axis. Consequently no single transverse section of the latter showed the central cavity completely replaced by the diaphragm; and, in a series of transverse sections passing from below upward through the node, sclerization of the cells seemed gradually to spread outward. As the result a sclerized sheath is formed round the reconstituted bundles of the stele. The sclerized band then ceases to be formed inside the bundles; and the sclerization still spreading outward, the band of sclerized cells comes to lie between the outer edges of the bundles and the traces. These latter are themselves moving slowly and steeply outward.

Soon after this phase has been attained, the innermost layer of the sclerized band, with all the tissue external to it, separates from the axis to form the free part of the leaf sheath. This innermost layer becomes the adaxial (morphologically upper) epidermis of the leaf sheath. At the level at which this separation took place, the outer part of the axial tissues was, in all my specimens of large axes, in a highly immature condition. Its cells had thin walls and were almost completely filled by large, deeply staining nuclei. This is, in fact, the base of the internode, which in *Equisetum* is the region where cell differentiation is longest deferred. Since all the tissues vertically

above the cortex and vallecular canals of the internode below have become separated off as part of the leaf sheath, the axis suddenly becomes much narrower above the diaphragm. As the leaf sheath widens, the stem again widens; epidermis, green parenchyma, and other tissues are again gradually differentiated; and the stem once more attains its ordinary internodal size and appearance.

Smaller aerial axes

In smaller stems definite inwardly projecting teeth of hypodermal fibers do not develop under the ribs and furrows. In the internodes the central cavity is still very wide, occupying usually from two-thirds to four-fifths of the whole axis; but relatively as well as actually it is smaller than in the largest axes. The vertical extent of the nodal xylem is markedly less, and the protoxylem of the trace seems to pass out more rapidly, pursuing a much less oblique upward course through the bundles. The amount of persistent tissue just below the diaphragm is relatively greater in these small stems. The diaphragm, too, seems often to be somewhat raised at the center of the axis, so that it has the shape of a low dome. A series of longitudinal sections passing through a cone, the peduncle of the latter, and the uppermost vegetative node revealed the presence in this specimen of a small rounded protuberance of parenchyma, seated on the central highest part of the low dome-shaped diaphragm. In the smaller stems also, the axial protoxylem of successive internodes is completely discontinuous. In the cone-bearing specimen just mentioned, the tissues, even immediately above the diaphragm, were fully mature; but there was a vertical distance of about 0.5 mm. from which protoxylem was absent, the only axial xylem present being nodal tracheids.

In the smaller stems, at least in my specimens, the branches attained a relatively greater development. Two cases were observed in which the tip of a branch just broke through the leaf sheath, although even in these cases they developed no further; for in none of my specimens were there any of those simple branches recorded by Schaffner in his diagnosis (**4**, p. 21). In other cases the leaf sheath bulged markedly outward and the exterior of the axis inside it was deeply indented opposite the dormant branches, so that the

latter could fit in between stem and leaf. In several cases the axis became for a time oval or irregularly heart-shaped, as seen in transverse section, owing to the pressure of the branches.

It is curious that at the level of the four nodes of the largest stems —those in which there were thirty or more bundles—only five branches were initiated, two of the nodes showing no dormant branches. At the four nodes with from fourteen to twenty-three bundles, nine branches were initiated, each node showing at least one dormant branch; some two, and some three. At the level of each of the nodes of the two smallest stems, with eight and ten bundles respectively, a single branch was initiated.

Discussion

This short account of the anatomy of *Equisetum kansanum* supports the view that this plant is specifically distinct from *E. laevigatum*. Anatomically *E. kansanum* appears to be nearer to *E. hyemale* and to *E. trachyodon* than to *E. laevigatum*. The similarity to *E. hyemale* is more particularly to the variety *doellii* (**2**, pl. xxix, fig. 19), since in the latter there is a rigid sheath of small cells separating outer and inner ground tissue. But of all the species the anatomy of which has been described, it is *E. trachyodon* that anatomically comes nearest to the larger axes of *E. kansanum*. The form of the sheath separating inner and outer ground tissue, the narrow oval outline of the bundles with their long axes directed radially, even the triangular form of the projecting teeth of hypodermal fibers under the ribs of the stem (as seen in transverse section of the axis) and the presence under some bundles of the larger stems of a radial row of cells with slightly thickened walls leading from the inwardly directed apex of the tooth to the periphery of the bundle,—all these can be seen in MILDE's figures of *E. trachyodon* (**2**, pl. xxxii, figs. 14, 18). Again, MILDE figures a bundle (pl. xxxiii, fig. 15) in which only one of the lateral bands of metaxylem has developed, an irregularity noted as occurring locally in *E. kansanum*. However, in *E. kansanum* the central cavity occupies an even larger proportion of the whole axis than in *E. hyemale* and in *E. trachyodon;* and the ribs of the stem of *E. kansanum* are slightly convex, and not, as in the other two species, concave with two lateral angles.

The smaller stems of *E. kansanum*, those with from eight to twenty-three bundles, do not show the same similarity to the stems of *E. trachyodon*, since there are in them no inwardly projecting teeth of hypodermal fibers or rows of cells with thickened walls running from these to the bundles. This is curious, because in size *E. trachyodon*—with eight to fourteen bundles and ribs—comes nearer to the smaller than to the larger axes of *E. kansanum*.

In 1867 MILDE, while maintaining that *E. trachyodon* was a good species in the same sense as were *E. ramosissimum*, *E. variegatum*, and *E. scirpoides*, admitted that it certainly passed over into *E. hyemale;* and he mentioned the variety *doellii* of *E. hyemale* as being such a transitional form. In describing var. *doellii* itself, however, MILDE, while repeating the statement that this variety represented a transition (*Übergang*) to *E. trachyodon*, added: "it can, nevertheless, be distinguished from *E. trachyodon*, in company with which it alone occurs, by its more vigorous growth, narrower ribs, . . . by the siliceous ornamentation of the epidermis of the stem and by the form of the leaf sheath, after the shedding of its teeth" (**2**, pp. 519, 563). In 1930 SCHAFFNER stated that he was unable to come to a conclusion as to the specific validity or hybrid nature of *E. trachyodon*, but that there were forms going under varietal names of *E. hyemale* which seemed to have their real affinity with *E. trachyodon;* and that when these were grouped together they made a respectable species of wide distribution in the northern part of the north temperate zone (**7**, p. 101). In 1932 SCHAFFNER, whose knowledge of these species of *Equisetum* in their natural habitat is probably unrivaled, took the step of placing *E. trachyodon*, including var. *doellii*, with *E. variegatum*, *E. nelsoni*, and *E. scirpoides*, in his Equiseta pusilla (**8**). This group, he believes, separated from the lower Equiseta hiberna "quite early in the evolutionary history, there being no direct living transition species" (**6**, p. 14). SCHAFFNER'S group of Equiseta hiberna includes *E. hyemale*, *E. ramosissimum*, *E. debile*, *E. laevigatum*, *E. praealtum* (= *E. robustum*), *E. moorei*, and *E. myriochaetum*.[6] In 1912, when SCHAFFNER first made *E.*

[6] In 1925 SCHAFFNER placed *E. myriochaetum* among his Equiseta primitiva; later he transferred it to the Equiseta hiberna, considering it as the lowest species of that group (**5**, **8**).

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kansanum into a separate species, he noted that in size and manner of growth it was closely allied to *E. hyemale* and to the larger variety of *E. robustum* (**4**, p. 21); and in 1925 he placed *E. kansanum* and *E. funstoni* at the two ends of a forking line originating farther back than the point of division of the two groups which, in his publications since 1930, he has distinguished as Equiseta hiberna and Equiseta pusilla (**5**, pl. iii; **8**, p. 14). In 1930 he held that *E. kansanum* was probably derived from *E. laevigatum* (**6**, p. 16). In view of the fact that, until the date of its recognition as a separate species, the former was usually regarded as an annual form of the latter, this is not surprising. While anatomical characters cannot by themselves claim to be decisive in regard to systematic position, and still less in regard to the bewildering problems of phylogeny, the anatomy of *E. kansanum* does suggest the possibility that the nearest affinity of this species may be either with *E. trachyodon* or with *E. hyemale*, through a somewhat larger form than the typical *E. trachyodon*, that is, through the form known to MILDE as var. *doellii* of *E. hyemale*, a variety which SCHAFFNER places in *E. trachyodon*.

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CHROMOSOME NUMBERS IN ROOT NODULES AND ROOT TIPS OF CERTAIN LEGUMINOSAE[1]

LOUISE WIPF

(WITH THIRTY-EIGHT FIGURES)

Introduction

An earlier paper (**56**) reported the chromosome numbers in infected cells of root nodules of red clover, common vetch, and garden pea to be twice the typical somatic number for these species. Further examinations have been made to determine: (1) whether a similar relationship holds in other species; (2) whether it holds consistently in genera presenting polyploid series; (3) the chromosome condition in plants in which nitrogen fixation has been substantially inhibited either in the presence of ineffective strains of *Rhizobium*, the presence of combined nitrogen in the nutrient, or by treatment with hydrogen or with carbon monoxide.

Chromosome numbers associated with bacterial infection in root nodules have heretofore received little attention. FRED (**19**) noted that the mitotic figures in the cells of the nodules of some fifty leguminous plants, and particularly of the white lupine (**20**), "are larger, very irregular, and not well marked, with uneven number of chromosomes" as compared with the cells of the root tips. This irregularity of chromosome numbers suggested that the nodular tissues may be analogous to diseased tissues of animals. DANGEARD (**14**) described enlarged nuclei in the infected cells of several of the Leguminosae. His chromosome determinations were only approximate, and no mention was made of a regularly increased number. He showed, however, a polar view of an equatorial plate with 26 chromosomes from a nodule of *Trifolium pratense*. This, as now

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known, is approximately the $4n$ number, 28. MILOVIDOV (**39**) found 42 chromosomes in cells of the cortex of the nodule of *Lupinus mutabilis*. LECHTOVA-TRNKA (**35**) reported approximately 16 as the chromosome number in nodular tissues of both *Sophora moorcroftiana* and *Robinia viscosa*.

Material and methods

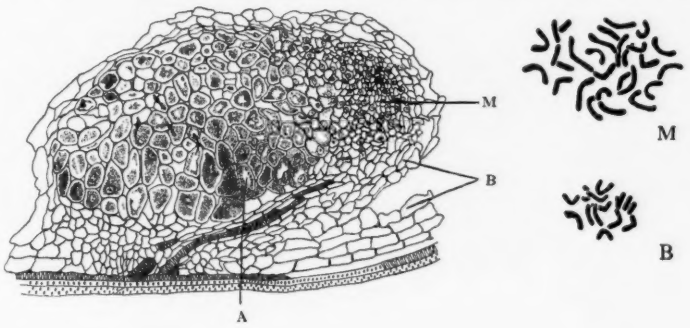
The nodules and root tips used in these examinations were obtained from plants growing in both controlled and natural environments. Controlled conditions involved the addition of Crone's nutrient solution and of an inoculum of the specific *Rhizobium* to the disinfected seeds at the time of planting. The inhibited plants were grown in sterilized soil and pots in the greenhouses of the department of agricultural bacteriology, University of Wisconsin, by Professor P. W. WILSON and co-workers. The *Melilotus* species were grown in the greenhouses of the department of genetics by Professor W. K. SMITH and those of *Medicago* by Professors R. A. BRINK and D. C. COOPER. The *Trifolium* series was obtained from plants grown by Dr. OLIVER SMITH of the U.S. Department of Agriculture. The plants, when nodules were at varying stages of maturity, were removed from the pots in which they were growing, and the exposed nodules and root tips were collected and fixed. Other material was obtained from plants of various species growing in the field. Chance inoculation had occurred in these, as well as in the plants of *Medicago*, *Melilotus*, and *Trifolium*.

Some material was fixed in Karpetschenko's modification of Navashin's fluid, imbedded in paraffin, and sectioned at thicknesses of 8–15 μ. Most of the nodules, however, were fixed in Carnoy's alcohol-acetic acid-chloroform solution and used for aceto-carmine smears. The latter method proved satisfactory for the determination of chromosome numbers in the nodules. Mitotic spindles in the meristematic region of the nodule are variously oriented. A higher percentage of polar views of equatorial plates is obtained by use of the smear technique than is possible in sectioned material. Either the apical meristematic portion of the nodule (fig. 1*M*) or the whole nodule, if in an early stage of development, was crushed in a drop of the stain and examined for division figures. This method was used

also for some root tips, but transverse sections of imbedded material usually gave a greater number of polar views of equatorial plates.

Although the somatic chromosome numbers for the majority of the species studied had previously been reported (**47, 48, 49, 23, 24, 25, 26, 38, 44**), a determination of the number was made in the root tips of each individual nodule-bearing plant.

Plants from which material used in this study was obtained may be classified in three groups: (1) thirty-one diploid species belonging to six genera (table 1); (2) plants belonging to three genera, each in-



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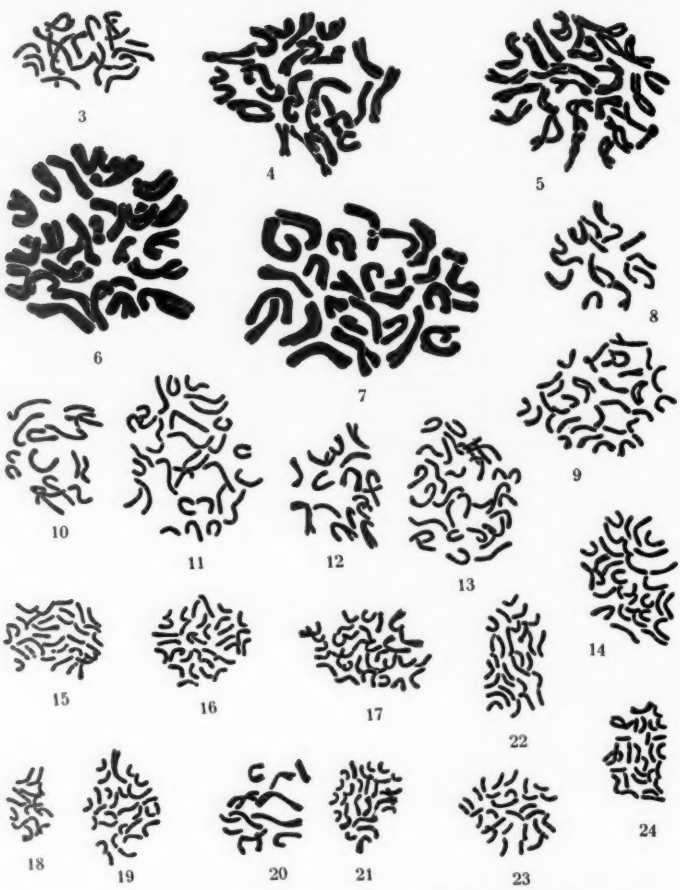
FIGS. 1, 2.—*Trifolium pratense*. Fig. 1, longisection through root nodule: *A*, older cells packed with bacteria; *B*, uninfected cortical cells; *M*, meristematic portion of infected area. ×80. Fig. 2, polar views of equatorial plates: *M*, infected cell from meristematic region, 28 chromosomes; *B*, uninfected cortical cell, 14 chromosomes. ×1580.

cluding a polyploid series (table 2); (3) plants known to be hindered in the fixation of free nitrogen by any of the means just mentioned (table 3).

Observations

DIPLOID SPECIES.—The numbers of chromosomes on equatorial plates of dividing infected cells in root nodules of thirty-one leguminous species, including members of the genera *Lathyrus*, *Medicago*, *Melilotus*, *Pisum*, *Trifolium*, and *Vicia* (figs. 3–24), were determined and compared with the numbers present in root tips of the same plants (table 1). In each instance the chromosome number in the infected nodular cells was twice that present in root tip cells.



FIGS. 3–24.—Chromosomes (numbers in parentheses) from root tip cells and infected nodular cells of diploid plants. Fig. 3, *Vicia angustifolia*, nodular cell (24). Fig. 4, *Pisum sativum*, nodular cell (28). Fig. 5, *Lathyrus odoratus*, nodular cell (28). Fig. 6, *L. ochroleucus*, nodular cell (28). Fig. 7, *L. latifolius*, nodular cell (28). Fig. 8, *Melilotus altissima*, root tip cell (16). Fig. 9, *M. altissima*, nodular cell (32). Fig. 10, *M. caspia*, root tip cell (16). Fig. 11, *M. caspia*, nodular cell (32). Fig. 12, *M. gracilis*, root tip cell (16). Fig. 13, *M. gracilis*, nodular cell (32). Fig. 14, *M. alba* (diploid), nodular cell (32). Fig. 15, *Medicago arabica*, nodular cell (32). Fig. 16, *M. lupulina*, nodular cell (32). Fig. 17, *M.* sp., nodular cell (32). Fig. 18, *Trifolium agrarium*, root tip cell (14). Fig. 19, *T. agrarium*, nodular cell (28). Fig. 20, *T. resupinatum*, root tip cell (14). Fig. 21, *T. resupinatum*, nodular cell (28). Fig. 22, *T. procumbens*, nodular cell (28). Fig. 23, *T. glomeratum*, nodular cell (28). Fig. 24, *T. incarnatum*, nodular cell (28). ×1580.

TABLE 1

CHROMOSOME NUMBERS IN DIPLOID SPECIES

SPECIES	NORMAL NUMBERS		INVESTIGATOR	NUMBER IN INFECTED CELLS
	n	*2n*		
	ALFALFA CROSS-INOCULATION GROUP			
1. Medicago arabica All.	.....	16	Fryer (22), Chekhov (9)	32
2. M. lupulina L.	8	.....	Tischler (47)	32
	8	16	Fryer (22)	
	.....	16	Ghimpu (27, 28), Chekhov (9)	
3. M. platycarpa Trautv.	8	.....	Tischler (47)	32
	.....	16	Fryer (22)	
4. M. ruthenica Trautv.	.....	16	Fryer (22)	32
5. M. sativa L.	16	.....	Tischler (47), Kawakami (32)	64
	16	32	Reeves (42)	
	.....	32	Elders (15), Fryer (22), Ghimpu (27, 28), Chekhov (9), Oppenheimer (41)	
6. M. sp.	.....	16	Brink (unpublished) *l.c.*	32
7. Melilotus alba Desr.	8	.....	Cooper (12)	32
	8	16	Castetter (7, 8)	
	.....	16	Elders (15), Fryer (22), Chekhov (9), Clark (11)	
8. M. altissima Thuill.	.....	16	*l.c.*	32
9. M. caspia Gruner	.....	16	*l.c.*	32
10. M. dentata Pers.	.....	16	Chekhov (9)	32
11. M. gracilis DC.	.....	16	*l.c.*	32
12. M. officinalis Lam.	8	.....	Cooper (12)	32
	.....	16	Elders (15), Fryer (22), Chekhov (9), Clarke (11)	
13. M. segetalis Ser.	.....	16	Clarke (10, 11)	32
14. M. speciosa Dur.	.....	16	Clarke (11)	32
15. M. suaveolens Ledeb.	.....	16	Chekhov (9)	32
16. M. sulcata Desf.	.....	16	Fryer (22), Chekhov (9), Clarke (10, 11)	32
17. M. wolgica Poir.	.....	16	Chekhov (9), Clarke (10)	32
	CLOVER CROSS-INOCULATION GROUP			
18. Trifolium agrarium L.	.....	14	*l.c.*	28
19. T. dubium Sibth.	.....	28	*l.c.*	56
20. T. glomeratum L.	7	.....	Bleier (3)	28
	.....	16	Wexelsen (50)	

TABLE 1—*Continued*

Species	Normal numbers		Investigator	Number in infected cells
	n	2*n*		
	Clover cross-inoculation group—*Continued*			
21. T. incarnatum L.	8	.....	Bleier (3)	28
	.....	14	Karpetschenko (31), Wexelsen (50)	
	.....	(14) 16	Oppenheimer (41)	
22. T. involucratum Dulac	.....	28	*l.c.*	56
23. T. pratense L.	7	.....	Kawakami (32)	28
	7	14	Bleier (3)	
	.....	14	Karpetschenko (31), Wexelsen (50)	
	.....	14 (24)	Oppenheimer (41)	
24. T. procumbens L.	.....	14	Karpetschenko (31)	28
25. T. repens L.	16	.....	Kawakami (32)	64
	14	.....	Bleier (3)	
	.....	16	Erith (16)	
	.....	32	Karpetschenko (31), Wexelsen (50)	
	.....	(24) 28, 32	Oppenheimer (41)	
26. T. resupinatum L.	8	.....	Bleier (3)	28
	.....	16	Karpetschenko (31)	
	.....	14	*l.c.*	
	Pea cross-inoculation group			
27. Lathyrus latifolius L.	7	.....	Simonet (45)	28
	7	14	Winge (53), Fisk (17)	
28. L. ochroleucus Hook.	.....	14	Senn (43)	28
29. L. odoratus L.	7	14	Winge (53)	28
	7	.....	Kawakami (32), Simonet (45)	
30. Pisum sativum L.	7	14	Canon (6)	28
	7	.....	Winge (54)	
31. Vicia angustifolia L.	.....	12	Sveshnikova (46)	24

Occasionally, in an aceto-carmine smear, diploid as well as tetraploid cells were found in the meristematic region of a nodule. In such a case, diploid cells occurred in a group in which the cytoplasm was clear and there was no evidence of infection. A cross section through the meristem of a pea nodule showed diploid cells in the uninfected cortical region. Tetraploid cells predominated in the central infected region.

No variation from the 2:1 chromosome relationship between infected and uninfected cells was found in any of the plants examined. Aberrant features of mitosis, such as absence of a spindle, multipolar spindles, lagging chromosomes, or irregularities in chromosome number, were not observed in any case.

POLYPLOID SERIES.—Polyploid series were available within the genera *Melilotus*, *Medicago*, and *Trifolium* (table 2). An autotetraploid strain of *Melilotus alba* ($2n = 32$) had been obtained by ATWOOD (1) by means of heat treatment from the diploid *M. alba*

TABLE 2

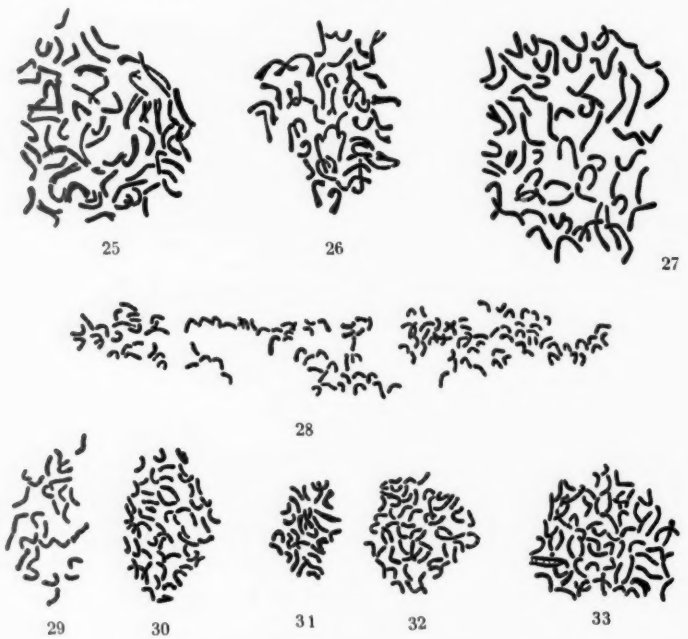
CHROMOSOME NUMBERS IN PLANTS OF POLYPLOID SERIES

SPECIES	NORMAL NUMBER ($2n$)	NUMBER IN INFECTED CELLS
Melilotus series		
alba (diploid)	16	32
alba (tetraploid)	32	64
Medicago series		
sp. (diploid)	16	32
sativa (tetraploid)	32	64
sativa × M. sp. (triploid)	24	48
sativa (octoploid)	64	128
Trifolium series		
agrarium	14	28
dubium	28	56
involucratum	28	56
repens	32	64

($2n = 16$). The chromosome number (32) in the root tips of this plant, although already tetraploid, was exactly half the number (64) found in the infected cells of the nodule (figs. 14, 25).

An undetermined species of *Medicago*, with the basic chromosome number $n = 8$ ($2n = 16$), was found to have the tetraploid number (32) in the infected cells of the nodule (fig. 17). *Medicago sativa* is a natural tetraploid ($2n = 32$) within this genus. The chromosome number in the infected cells of the nodule of this species is 64 (fig. 27). BRINK made a successful cross between *M. sativa* ($2n = 32$) and the undetermined species ($2n = 16$) just mentioned, and obtained a triploid plant with 24 chromosomes (unpublished). The number of chromosomes in the infected cells of the nodule of this triploid plant was found to be 48 (fig. 26). An artificially induced autotetraploid alfalfa with 64 chromosomes (octoploid in terms of

the basic number for the genus) has been obtained by COOPER (13). One hundred twenty-eight chromosomes are present in the infected cells of the nodule of this plant. An anaphase configuration in an infected cell (fig. 34) shows two groups, each with 128 chromosomes,



FIGS. 25–33.—Chromosomes from root tip cells and infected nodular cells of polyploid plants. Fig. 25, tetraploid *Melilotus alba*, nodular cell (64). Fig. 26, triploid plant, cross between *Medicago sativa* and *M.* sp., nodular cell (48). Fig. 27, *M. sativa*, nodular cell (64). Fig. 28, tetraploid *M. sativa*, nodular cell (128). ×1080. Fig. 29, *Trifolium dubium*, root tip cell (28). Fig. 30, *T. dubium*, nodular cell (56). Fig. 31, *T. involucratum*, root tip cell (28). Fig. 32, *T. involucratum*, nodular cell (56). Fig. 33, *T. repens*, nodular cell (64). ×1580.

on the spindle. Figure 28 shows one of these groups in which the chromosomes were so arranged that the number could be rather readily determined.

A natural polyploid series exists in *Trifolium*. Six of the nine species examined (table 1) show 14 chromosomes in root tip cells;

two—*T. dubium* Sibth. (fig. 29) and *T. involucratum* Dulac (fig. 31)—possess 28, and one (*T. repens* L.) has 32. Here again the chromosome number in the infected cells of a nodule is in each case twice the number present in root tips of the same plant (figs. 18–24, 29–33).

PLANTS WITH INHIBITED NITROGEN FIXATION.—Some *Rhizobium* strains (designated as "poor" or "ineffective") become parasitic

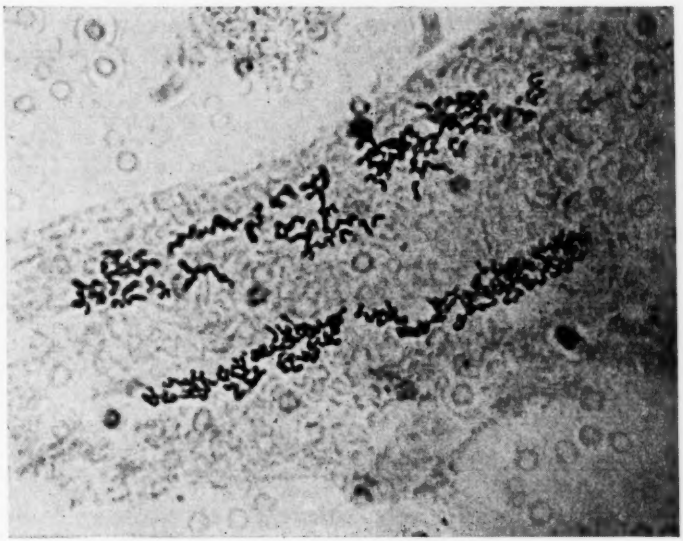


FIG. 34.—Anaphase in nodule of tetraploid *Medicago sativa;* 128 chromosomes (16*n*) passing to each pole; upper chromosome group shown in fig. 28.

rather than symbiotic upon the host plant (**18, 21**). When a plant is infected by a poor strain, an abundance of nodules may be formed scattered over the entire root system but fixation of free nitrogen does not occur. In nodules of *Pisum sativum* L. formed in consequence of inoculation with University of Wisconsin strain 311 (ineffective) of *Rhizobium leguminosarum* Frank, the chromosome complement of the infected cells was, as in previous cases, double the number in normal cells (table 3, fig. 35).

Seedlings of *Pisum sativum* were inoculated with an effective strain of *Rhizobium leguminosarum* (U. W. strain 317). A solution of

calcium nitrate was added to the soil in which the plants were growing at the time of inoculation and again each week thereafter for five weeks. When combined nitrogen is added to the nutrient, very few nodules are formed and the fixation process is inhibited (**21, 29**). The nodules produced after this treatment grew slowly, were few and small. Sampling was difficult because of the reduced number of nodules available, and mitotic figures were less numerous than in more rapidly growing nodules. The number of chromosomes present in the infected cells (fig. 36), however, was double that found in root tip cells.

TABLE 3

CHROMOSOME NUMBERS IN PLANTS WHOSE NITROGEN FIXATION IS INHIBITED

SPECIES	NORMAL NUMBER ($2n$)	NUMBER IN INFECTED CELLS
Ineffective strain of Rhizobium		
Pisum sativum inoculated U.W. strain 311	14	28
Nitrate ($CaNO_3$) added to nutrient		
P. sativum inoculated U.W. strain 317	14	28
Hydrogen		
Trifolium pratense (Al+NaOH H_2)	14	28
Carbon monoxide		
T. pratense (air+0.1% CO)	14	28

WILSON and UMBREIT (**52**) found that if hydrogen is supplied to the atmosphere surrounding the host plant, the fixation process is inhibited. In the experiments with which the present paper is concerned, the germinated seedlings of *Trifolium pratense* L. were inoculated at the time of planting. The plants were allowed to grow for three or four weeks, or until the second or third trifoliate leaf appeared, then hydrogen was substituted to a large extent for the air in the bottles in which they were growing. Once a week the plants were aerated and the gas was changed. Carbon dioxide was added when necessary. The chromosome number in the nodules under these conditions was the same as before treatment ($28 = 4n$, fig. 37).

Carbon monoxide, when added in like manner, similarly inhibits the fixation of nitrogen (private communication from P. W. WILSON and S. B. LEE). This gas was supplied to red clover seedlings. The

plants so treated showed a great decrease in nitrogen fixation. The infected cells from the nodules, however, were tetraploid ($28 = 4n$, fig. 38).

CHROMOSOME CHARACTERS.—Equatorial plates in infected cells (figs. 9, 11, 13) are larger, and the chromosomes are more compactly arranged, than in normal diploid cells (figs. 8, 10, 12). The individual chromosomes in infected cells closely resemble in form and size those in cells of root tips. A marked similarity can be noted be-

35

36

37

38

FIGS. 35–38.—Chromosomes from infected nodular cells of plants whose fixation of atmospheric nitrogen had been inhibited. Fig. 35, *Pisum sativum* after inoculation with ineffective strain of *Rhizobium* (U.W. strain 311); 28 chromosomes. Fig. 36, *P. sativum*, $CaNO_3$ added to nutrient (28). Fig. 37, *Trifolium pratense* grown in atmosphere containing added hydrogen (28). Fig. 38, *T. pratense* grown in atmosphere containing added carbon monoxide (28). ×1580.

tween the chromosomes on a normal somatic equatorial plate (fig. 8) and those on an equatorial plate in an infected cell (fig. 9) of the same species. In those instances in which a pair of chromosomes in a somatic equatorial plate can be identified with certainty because of some particular morphological feature, four similar chromosomes appear in equatorial plates in infected cells of a nodule on the same plant. An infected cell of *Pisum sativum* inoculated with an ineffective strain (fig. 35) shows four similar satellite chromosomes, whereas two such chromosomes are present in root tip cells. Although similar comparisons for each individual chromosome have not been

attempted, size, shape, and general appearance indicate that the complement of an infected cell results from a simple doubling of the normal complement.

The species whose normal chromosome numbers are here first reported are:

Melilotus altissima	$2n=16$, fig. 8
M. caspia	$2n=16$, fig. 10
M. gracilis	$2n=16$, fig. 12
Trifolium agrarium	$2n=14$, fig. 18
T. dubium	$2n=28$, fig. 29
T. involucratum	$2n=28$, fig. 31

Discussion

It appears from the results that the chromosome number in the infected cells of nodules of every plant studied is twice the number present in the uninfected root tip cells of the host. The same relationship exists in plants so treated as to inhibit the fixation of free nitrogen. No irregular mitotic phenomenon has been observed in any case.

The observations on diploid plants (those possessing twice the basic number for the genus) might seem to indicate that a certain chromosome number is necessary for this symbiotic relationship. When the polyploid series are considered, however, it appears that the absolute chromosome number is not the significant factor, but rather the 2:1 relationship between infected and uninfected tissue. It appears, too, that this relationship is not disturbed once the nodules are initiated, even though nitrogen fixation is partially or totally prevented. A study of the development of the nodule beginning with the cell or cells first infected, involving the recognition of the initiation of polyploidy and an observation of the multiplication of polyploid cells, may be expected to suggest the causal factor or factors concerned.

It has long been known that nuclear size and cell size are commonly, though not invariably, correlated with chromosome number (4). Meristematic infected nodular cells have approximately twice the volume of the uninfected (diploid) cells of the nodular cortex. As the infected cells of a young nodule mature, they become more or less gorged with rhizobia (fig. 1*A*). The nuclei of these mature host cells are in varying stages of disintegration.

Infected overgrowths of various types have been found to possess chromosome numbers differing from those typical for the species. Diploid, tetraploid, and octoploid cells have been observed in crown gall tissue of sugar beet (**55**) and of beet and tobacco (**36, 34**). Wide variation in chromosome numbers, ranging from haploid to tetraploid or even higher, are found in the carcinomatous tissue in the fowl, rat, and man (**36**). Abnormal tissues, such as the galls produced by parasites, contain tetraploid cells (**40**). Gall mites upset the meiotic divisions in *Lycium halimifolium*, resulting in many irregularities in the chromosome numbers (**33**).

Agents, including various chemical substances, heat, cold, x-ray, and wounding, have been used successfully to produce polyploidy in a number of forms. BLAKESLEE and AVERY (**2**) obtained plants with irregularly increased chromosome numbers by the application of colchicine to the seeds or seedlings. Buds from callous tissue in tomato and *Solanum* spp. formed tetraploid shoots (**30, 37**). It is possible either that tetraploidy was produced by the wounding, or that tetraploid cells were already present. WHITAKER (**51**) reports occasional tetraploidy in cells of nonparasitic tumors on grafted tobacco. WULFF (**57**), as well as earlier workers, has found "polysomaty" occurring naturally in the periblem of several genera of the Chenopodiaceae. This condition is reported also in *Cannabis sativa* (**5**). A preliminary examination of dividing cells in the root hair region of *Pisum sativum* showed a few scattered tetraploid figures in the cortex near the endodermis. The chromosomal condition in leguminous root nodules differs, so far as is now known, from that in other stimulated growths by the constant occurrence of the doubled number. It is not the mere doubling that seems to be most significant, however, rather is it the 2:1 chromosome balance that is established between infected and uninfected cells. This 2:1 ratio is so constant that it may well have some definite bearing upon the symbiotic relationship between the host plant and the infecting organism.

Summary

1. Infected cells of root nodules of thirty-one diploid species belonging to *Lathyrus, Medicago, Melilotus, Pisum, Trifolium*, and *Vicia* contain in each case the tetraploid number of chromosomes.

2. Uninfected cells in the nodular cortex possess the diploid number characteristic of the particular species.

3. The chromosome number in infected nodular cells in diploid and autotetraploid strains of *Melilotus alba* is in each case twice that characteristic of uninfected somatic cells of the same plant.

4. The chromosome number in infected cells of the nodules from diploid, triploid, tetraploid, and octoploid species and races of *Medicago* is in each case double that in root tip cells.

5. Infected nodular cells from plants of a natural polyploid series in *Trifolium* possess in each case twice the somatic number of chromosomes.

6. Inhibition of the fixation of free nitrogen by such conditions as infection with an ineffective strain of *Rhizobium*, the presence of combined nitrogen in the nutrient, or the presence of hydrogen or of carbon monoxide does not affect the chromosome numbers of the nodular cells. Infected cells regularly present twice the number found in uninfected cells.

7. There is definitely and consistently a 2:1 chromosome ratio between infected and uninfected cells of the root nodules on the leguminous plants examined.

8. Diploid chromosome numbers herein reported for the first time are: *Melilotus altissima* $2n = 16$, *M. caspia* $2n = 16$, *M. gracilis* $2n = 16$, *Trifolium agrarium* $2n = 14$, *T. dubium* $2n = 28$, and *T. involucratum* $2n = 28$.

The writer is indebted to Professors D. C. COOPER and C. E. ALLEN for kind advice and constructive criticism so generously given throughout these observations. Further acknowledgment is extended to Professor P. W. WILSON and Dean E. B. FRED for suggestions, material, and continued interest.

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PHYSIOLOGY OF LAZY CORN

JOHN SHAFER, JR.[1]

(WITH THREE FIGURES)

Introduction

One recessive gene, showing typical Mendelian inheritance, controls the laziness of lazy corn. If that gene is present in homozygous condition it causes the plant to be lazy. Under field conditions lazy corn appears to be normal at first; it grows erect. At some time during its development (in these experiments, when it has reached a height of 1 to 2 feet) it begins to lean from the vertical, and this leaning becomes more and more pronounced until the plant is lying on the ground. The bending appears to occur entirely in the first two or three internodes above the ground; the roots remain firmly fixed in the soil. The plant continues growth along the ground with no apparent tendency to become erect (save in rare cases). Finally, it produces tassel and ear in the manner of ordinary corn.

VAN OVERBEEK (10) has shown that during the first few days of their development, lazy plants are normal in respect to geotropism. Then they lose the ability to respond and become ageotropic. Growth measurements of stems of partly mature corn plants, lazy and normal, indicate that the difference between the two sorts is at least partly one of growth, although JENKINS (5) has shown that laziness is probably due in part to mechanical weakness. Since growth differences between lazy and normal corn were evident, it was hoped that some abnormality in the amount or distribution of the growth hormone would be found to explain the abnormal geotropic response of lazy corn.

Experimentation

GROWTH MEASUREMENTS

Numerous measurements were made of the growth of stems of nearly mature corn. A number of evenly spaced sewing needles were

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soldered to a piece of brass, forming a "comb" with which a stem could be marked by pricking through the inclosing leaf sheaths (**4**). The distance between the original needle marks being established, final measurements would disclose how much growth had taken place between the marks and in what region of the original stem it had occurred. The distance between needles averaged 0.76 mm. The holes produced by such needle pricks were apparent even after two weeks, except in very young, rapidly growing tissues. The final distances were measured with an ocular micrometer in a dissecting microscope, and were read to the nearest 0.05 mm. All marks for growth measurements were made at and just above nodal regions, and measurements were made from the external upper point of leaf insertion on the stem as the zero point, or node.

In both lazy and normal plants the growing zone was found to be 1 to 4 mm. above the insertion of the leaf (fig. 1). The growing zone varied from 0 to 10 mm. in length, with the average length of those measured about 3 mm. In the longer growing zones the upper parts had grown relatively slowly, however, most of the growth being in a zone of only a few millimeters. In very young nodal regions the growing zones were wider than in the older ones, and in the former growth was exceedingly rapid.

It was easy to induce growth in nodes of normal plants which would not ordinarily have grown more (fig. 2*A*, *C*). It was necessary only to place the plants horizontally, and nodes one or more internodes below those which grew in control plants would show distinct growth. This inauguration of growth in mature corn nodes agrees with the findings of earlier workers (**8, 9, 6**). They have stated that nodes of various grasses which have stopped growth begin to grow again when placed horizontally. SACHS reported considerable shortening of the upper sides of the up-bending nodes, whereas JOST found little or no shortening. The present work indicates that both conditions exist, even in the same plant (fig. 2*A*). JOST showed further that the sides of maize stems grow an amount equal roughly to the average of top and bottom growth. This, too, was confirmed by a few measurements made during the present study. The induced growth, occurring mainly in the lower side of the stem, caused the plants to bend upward. The new tissue formed a visible wedge, dis-

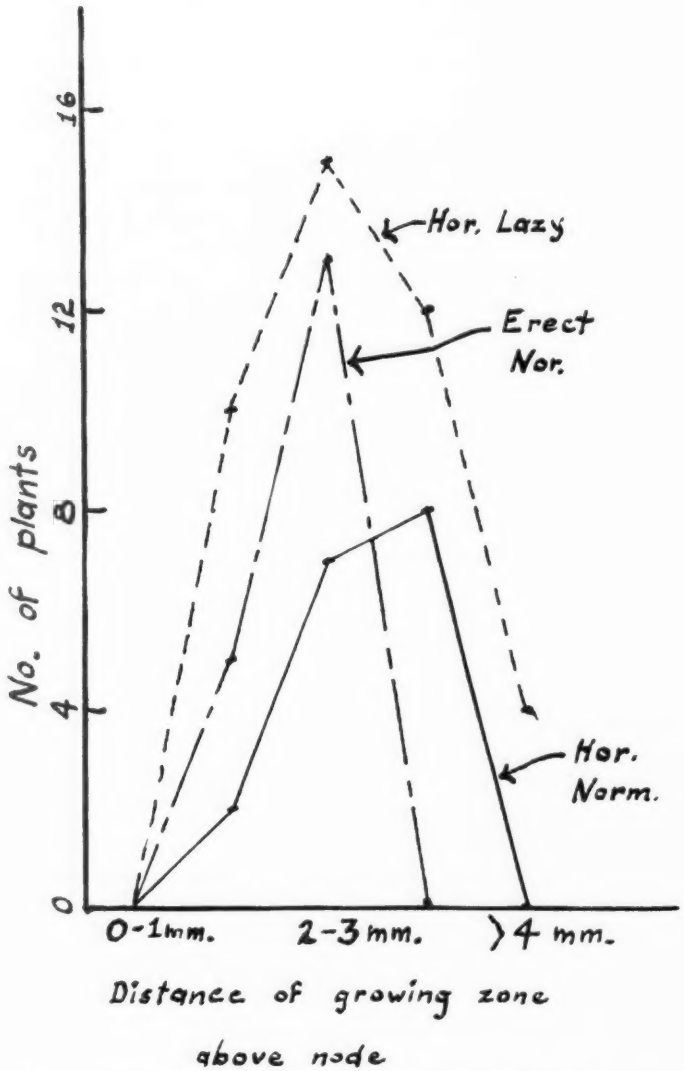

FIG. 1.—Frequency curve showing distances of lower edges of growing zones above their nodes. Curves are for: horizontal lazy; horizontal normal; and erect normal plants.

tinct in appearance from the ordinary stem tissue, and having its broad edge downward.

Microscopic examination of a few hand sections of this new tissue, kindly prepared by Mr. M. W. QUIMBY, indicated that the epidermal cells enlarge, as SACHS (**9**) states; that the parenchyma

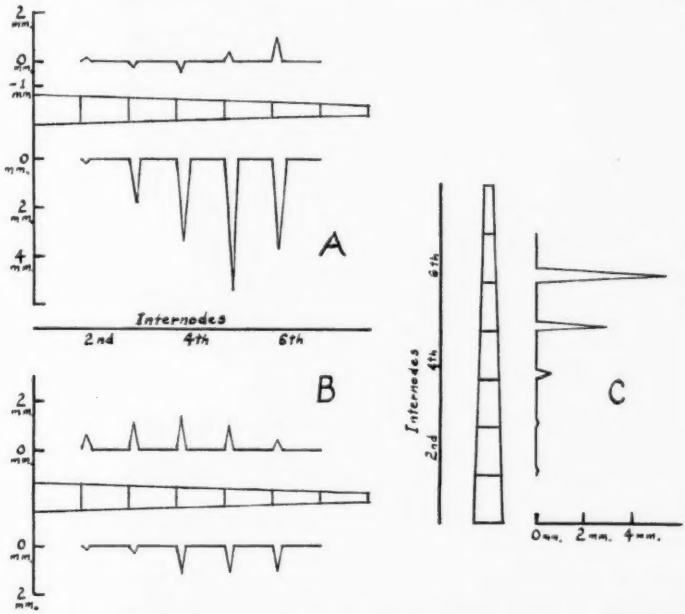

FIG. 2.—Schematic representation of growth of corn stems (shown with only seven internodes). Average amount of growth of each internode shown in mm. (on ordinate in *A* and *B;* on abscissa in *C*). *A*, horizontal normal; *B*, horizontal lazy; *C*, erect normal.

cells of the pith do not enlarge (SACHS states that they do) and must therefore multiply; that the newly formed vascular strands are much larger than normal, owing to the presence of many sclerenchymatous fibers.

Several wedges of new tissue were found at the bases of prostrate lazy plants, but with the broad edge up (as if the lazy plant had grown down). A horizontal position had no effect on the growth of

lazy corn, so far as could be determined (fig. 2*B*); one would expect this from the lack of response of lazy to gravity. There was some indication that lazy nodes continued growing after comparable normal ones had ceased growth (fig. 2*B*, *C*).

In some cases the holes on the upper side of horizontal normal plants were closer together after the plants had bent up than were the original needle pricks, showing that growth of the bottom side had actually compressed the top side (fig. 2*A*). In some such cases the top side appeared wrinkled. More often there was no sign of compression of the top side of horizontal normal plants, the stems having remained about the same size or even grown a little.

No method was found to induce growth in recently mature lazy nodes. The external application of 0.2 per cent heteroauxin-lanolin paste to such nodes had absolutely no effect. On normals such paste occasionally caused slight growth in thickness, but never any measurable growth in length.

Auxin production

There proved to be no obvious correlation between the amount of auxin obtained by diffusion from a coleoptile and the normal or lazy nature of the older plant. Seeds of such genetic nature that they would produce about 50 per cent lazy and 50 per cent normal plants were germinated in the dark. The coleoptile tips were cut off when 1–2 cm. above the ground, and were placed on small agar blocks, one tip to a block. After time had been allowed for the outward diffusion of the auxin, the blocks were tested for auxin content by the standard *Avena* test (**12**). The yield of auxin varied considerably, probably depending in part on the age and height of the plants from which the coleoptiles were cut. After removal of the coleoptiles, the plants were placed in the greenhouse to grow until they were old enough for the lazies to be ageotropic. Then they were tested by being placed horizontally (**10**); finally, the amount of bending of each was compared with the auxin yield of its coleoptile. The average yield of auxin by coleoptile tips of lazy plants was slightly higher than that by tips of normal plants.

For each of the experiments on the production of auxin by coleoptile tips, the correlation coefficient has been calculated. Nine

such experiments were performed; of these, eight showed so little correlation between auxin yield of tips and laziness of older plants that the odds in favor of the significance of such correlation were well below 9:1. In one case the odds in favor of significance were greater than 19:1. In view of the eight experiments showing no correlation, this one case of rather definite correlation must have been due to chance. It is concluded that there is no correlation between auxin yield of coleoptile tips and laziness of older plants. Since VAN OVERBEEK (10) reported young seedlings of lazy corn to be normally geotropic, this is the expected result.

Efforts to obtain auxin by diffusion, in a manner similar to that mentioned in the preceding paragraph, failed when applied to first internodes (mesocotyls) of two-weeks-old seedlings, to stems and leaf bases of such seedlings, and to stem sections of nearly mature plants. Either there is little auxin in such tissues or the destruction at the cut surfaces is high. The presence in the auxin-receiving agar blocks of various substances had no effect; sucrose, cysteine, ascorbic acid, and indole(3)propionic acid were tried.

AUXIN TRANSPORT

Some transport tests gave instructive although not conclusive results. Transport of auxin through first internodes was never found. Even concentrations of auxin equivalent to 450° oat curvature, placed on top of an internode piece 10 mm. long for 110 minutes, or 675° auxin on an internode piece 4 mm. long for 60 minutes, were not sufficient to produce apparent transport. That is, no measurable amount of auxin moved from a block of agar containing auxin of such a concentration and placed on top of the internode section, through the section and into the block of plain agar on which the section was standing.

Sometimes transport was obtained through tender growing zones of stems of semi-mature plants. Probably the condition determining whether or not there would be such transport was the stage of development of the section of tissue used, but that is not certain. In some transport experiments there were used vertical sections of stems, or slabs cut from stems and placed horizontally; these types of tests were abandoned in favor of a combination type. In the

preparation of sections for all the transport experiments, care was taken to select similar appearing plants and to choose from them sections that were apparently comparable.

For the combination type of test, sections 1 cm. long of growing zones were cut and clamped firmly in a horizontal position. The erstwhile bottom end was divided into two equal parts by the horizontal insertion of a piece of razor blade. Above and below this were placed plain agar blocks, each resting flat against its half of the cut surface. On the top end of the section was placed agar containing auxin. The concentration of this auxin was varied in the direction that seemed to promise the best transport results. From such an experiment some measure of the lateral transport is obtained by the relative amounts of auxin in the upper and lower agar blocks on the basal end; likewise the total transport through the stem section is determinable.

In general these tests showed greater transport by lazy than by normal stems (table 1 and figure 3). Transport by the lazy was not always greater than that by the normal which had been selected as a check; this might be expected from the impossibility of getting uniform, comparable tissue. The plants used were nearly always from seeds from the same ear; but since this seed was not inbred, the plants must have varied much in spite of the care used to select similar ones. At times there was no appreciable transport; occasionally normal transported more than lazy; usually lazy transported the more—nearly twice as much, on the average (table 1 and figure 3).

As for the lateral transport determined from these same experiments (table 1), normal stem sections showed more auxin in the lower agar block six times, more in the upper one twice, and essentially the same amount in each block nine times; of these nine cases, six were due to insignificantly small transport. The ratio of auxin in the lower blocks to that in the upper was about 3:2. Lazy, out of seventeen experiments, showed more auxin in the lower block five times, more in the upper one seven times, and the same amount in each five times, of which two were due to lack of transport. The ratio of bottom to top was about 4:5.

The greater amounts of auxin in the lower blocks of normal corn

is to be expected from similar work on other plants. Several investigators (3, 7, 2, 1) have reported that in horizontally placed, negatively geotropic plant parts auxin becomes more concentrated in the lower side. The ratio of auxin in lower halves of plant parts to that in upper halves is reported variously as between 54:46 and

TABLE 1

AUXIN TRANSPORT BY GROWING ZONES OF SEMI-MATURE LAZY AND NORMAL PLANTS

Experiment no.	Lazy*			Normal*		
	Top	Bottom	Average	Top	Bottom	Average
1	4.3	4.3	4.3	0.5	0.3	0.4
2	3.3	5.8	4.6	− 0.3	− 0.2	− 0.25
3	1.8	− 0.3	0.75	4.3	4.6	4.5
4	6.0	2.5	4.25	1.2	2.5	1.8
5	7.2	3.2	5.25	1.5	1.4	1.5
6	3.3	3.0	3.2	3.8	2.2	3.0
7	2.5	3.5	3.0	2.2	4.5	3.3
8	1.4	4.8	3.1	1.7	1.5	1.6
9	4.3	1.3	2.8	1.5	2.7	2.0
10	1.5	2.2	1.8	0.2	0.4	0.3
11	0.4	0.2	0.3	− 1.0	0.2	− 0.4
12	16.2	9.0	12.6	0.25	1.3	0.75
13	2.0	2.3	2.1	1.4	− 0.3	0.5
14	− 0.2	0.0	− 0.1	0.1	0.2	0.1
15	3.2	7.3	5.2	− 0.4	− 0.9	− 0.7
16	0.8	0.0	0.4	4.1	7.1	5.9
17	1.8	− 0.3	0.75	4.3	8.9	6.6
Total	59.8	48.8	54.3	25.4	36.4	30.9

* Nearly all these figures are the average of curvatures of six or twelve test plants.

75:25. The ratio just reported for sections of normal corn stem falls within this range (*ca.* 60:40). The data in table 1 show that there is no downward transport of auxin as it passes through the horizontal sections of lazy stem. On the contrary they suggest that there may be some upward transport. This in turn suggests that semi-mature lazy plants are positively geotropic. The data are insufficient to prove this, but VAN OVERBEEK (11) has recently obtained corroborative evidence.

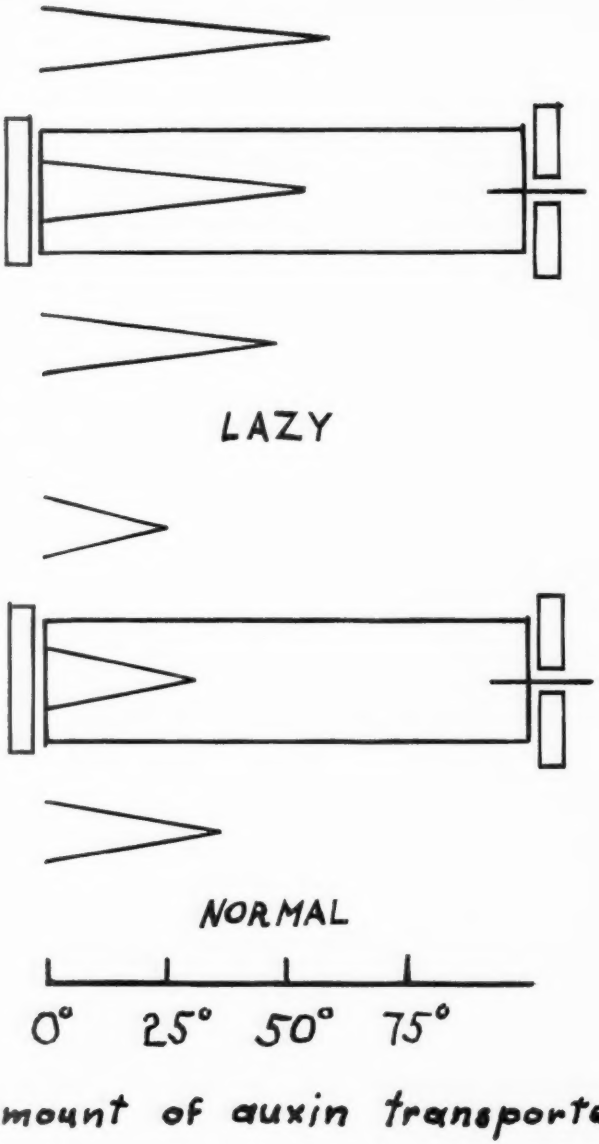

FIG. 3.—Schematic representation of auxin transport through sections of corn stems. Diagrams are idealized drawings of experimental set-up. Amount of auxin transported shown by heights of wedges. Transport through top and bottom halves of sections, and average of these, shown. Transport given as total degrees of oat curvature (see table 1).

Temperature and laziness

Efforts to study the effects on laziness of light intensity and duration failed because of temperature variations; consequently temperature effects were investigated. Three chambers were prepared, of which one was kept at about 10° C. (range 8.5° to 10.5°); one at about 22° C. (21° to 24°); the third at about 30° C. (29° to 33°, but dropped twice to 23° for periods of a few hours). The light in each chamber was artificial, coming from a 150 watt Mazda bulb about 1.5 feet from the plants. The light intensity was low but was nearly the same in all three chambers, having been adjusted with the aid of a Weston light-meter. Since the light came from the side and the plants were all bent sideways, phototropic response offered the most simple test for laziness, requiring only that the plants be turned at the start of a test to be bending away from the light. Such a test, depending on the amount of bending back toward the light, seemed as satisfactory as Van Overbeek's geotropic test (**10**). To be sure, it is not certain that lazy plants become aphototropic and ageotropic at the same time. However, the plants in these tests were aphototropic when about of a size which should have been ageotropic. Moreover, Van Overbeek's geotropic test for lazy would not work in ordinary light unless the plants were both ageotropic and aphototropic.[2] In these temperature experiments both lazy and normal plants from 100 per cent lazy and normal lots of seed of different strains were tested. Although the temperature control was not exceedingly exact, yet the results are fairly distinct and convincing.

In general, the higher the temperature the earlier the lazy plants became lazy. When grown at 30° C. they became lazy about three days after germination. At 22° it took three and one-half to four days for laziness to appear. At 10° the plants remained partly normal until eight or ten days after germination. All lots became lazy when they were of about the same height, regardless of the number of days between germination and the attainment of that

[2] Tests recently carried out with twenty-three horizontal lazy seedlings and as many erect ones of the same age with unilateral light showed only a few of the plants beginning to bend up in three days; also, a few were beginning to bend toward the light. This further indicates that lazies become aphototropic and ageotropic at about the same time.

height. Apparently temperature, per se, does not affect laziness; the effect is through an influence on the growth rate. It would seem that a lazy plant becomes ageotropic and aphototropic at a rather definite stage of development.

Discussion

Growth measurements of semi-mature lazy and normal corn stems (*Zea mays* L.) showed that both forms grow only in definite regions of the stem, which regions begin 1–4 mm. above the insertion point of the leaf. The growing regions vary in length from nothing to about 1 cm., being longer in the younger, more rapidly growing parts of a stem. The growth of normal stems is strongly influenced by gravity, even to the extent of recommencing if it has ceased not too long since. In such cases of induced growth it is the under side which grows, the upper side remaining the same length as before, or in some cases being compressed. The growth of lazy stems seems unaffected by gravity, although there is some indication that it persists longer than in normals.

The production of auxin by coleoptile tips bears no relationship to the lazy or normal nature of mature plants. Since seedling plants of lazy show normal geotropism, this is to be expected.

Transport tests in which sections of semi-mature stems were used suggest that lazy transports more auxin than normal corn. Of more importance is the lateral distribution of this auxin. In normal corn more of it is transported in the lower side of the stem, the ratio of bottom to top being about 3:2. This agrees with the findings of other workers in regard to other negatively geotropic plant parts. On the other hand, the auxin in lazy stems does not accumulate in the bottom of the stem but seems to move toward the top side, the ratio of bottom to top being 4:5. This suggests that lazy corn is positively geotropic.

Temperature studies, in which phototropism was used as a test for lazy, indicate that the time of appearance of laziness is definitely affected by temperature. The higher the temperature the shorter the time between the germination of the seed and the appearance of laziness. Regardless of temperature, however, the plants become lazy when of a fairly definite size. Hence it seems probable that they

become lazy at a definite stage of growth, and that temperature affects laziness indirectly, through its effect on the growth rate.

Summary

1. Corn stems grow only in definite intercalary regions which are 1 to 4 mm. above the leaf insertion points, and are 0 to 1 cm. long.

2. Growth can be induced in recently mature normal nodes by placing the plants in a horizontal position. Such a position has no effect on the growth of lazy stems.

3. Lazy stems tend to grow for a longer time than do normal ones.

4. Auxin yield of coleoptile tips is independent of laziness.

5. Auxin is transported more readily by lazy than by normal stems.

6. Auxin is so redistributed in horizontal normal stems that about 60 per cent of it moves to the lower side. This redistribution is reversed in lazy stems, so that about 55 per cent moves in the upper half.

7. Low temperature delays the time of appearance of laziness, but probably only by slowing up the growth rate.

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LOCALIZATION OF PHOTOPERIODIC PERCEPTION IN HELIANTHUS TUBEROSUS[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 502

KARL C. HAMNER AND ELBERT M. LONG

(WITH FOUR FIGURES)

Introduction

Among the many papers on photoperiodism published within the last few years, convincing evidence for photoperiodic perception by leaves in a wide range of plants has been presented (1–9).

On the basis of experiments with the Jerusalem artichoke, *Helianthus tuberosus* L., ZIMMERMAN and HITCHCOCK (11) found that tubers were formed by the plants maintained continuously on conditions of short day, and by those capped with the black bags, but none were formed on the plants exposed to the long days of summer. They describe the three sets of conditions as follows: "Under normal day length during summer months; plants covered with a black cloth from 4:30 P.M. to 9 A.M.; stem tips, only, capped with black cloth from 4:30 P.M. to 9 A.M." They concluded that "there is every indication that the growing stem tip has a regulatory influence on the development of underground stems and tubers. It seems likely that the regulators are chemical agents of a hormone-like nature, manufactured in the stem tip and sent to other parts of the plant where they can exert a controlling influence on the development of the underground portions."

Because of the amount of recent evidence, including that of FABIAN (3) dealing with tuber formation in *Ullucus tuberosus*, nearly all of which indicates that the leaves are the principal organs of photoperiodic perception, the following experiments were conducted to investigate the behavior of the Jerusalem artichoke.

[1] This investigation was aided in part by a grant to the University of Chicago from the Rockefeller Foundation.

Methods

One hundred tubers of *Helianthus tuberosus* L., each weighing about 50 gm., were selected for uniformity and planted singly in 8-inch pots in light garden soil. The soil was kept moist, and as soon as the tip of the first shoot appeared, all the pots were transferred to a greenhouse bench provided with supplementary illumination of Mazda light at an intensity at the soil surface of about 100 foot candles, from sundown until 2:00 A.M. The duration of the photoperiod on this bench (referred to as the long photoperiod bench) varied somewhat, but was never less than 18 hours. During their first month of growth, all the plants were fertilized by watering twice weekly with a modified, double strength, SHIVE's (**10**) R_2S_5 nutrient solution, to supplement the nutrients already present in the soil.

On April 18, when the plants were a little over two weeks old, they were segregated for various treatments. Six pots were transferred to a short photoperiod bench, which was covered regularly, except from 7:30 A.M. to 4:30 P.M., with a curtain of two layers of black sateen cloth, thus providing photoperiods of 9 hours and dark periods of 15 hours' duration. Another group of six pots was left on the long photoperiod bench, but one leaf was given short photoperiod by the use of small cylindrical cardboard boxes as described previously by HAMNER and BONNER (**4**). The terminal bud and young leaves of another group of six plants were given short photoperiod by an adaptation of the same method, as shown in figure 1. Cardboard boxes were placed in position over the tip of the plant at 5 P.M. and removed at 8 A.M. The boxes were about 3 inches in length, and expanding leaves which were less than 3 inches in length were included in the treatment. The stems were strong enough to support the weight of the lid of the box. A mixture of clay flour and castor oil was used to seal the space between the stem and the lid. The boxes were suspended in position by strings made fast overhead.

In a third group of six plants, only the terminal bud was placed on short photoperiod. Before the box covers were put in place all the young leaves were trimmed off with a sharp scalpel, except those 5 mm. or less in length. As new leaves expanded, they too were trimmed off, usually before they reached lengths of 1 cm. In order to make sure that this somewhat drastic treatment did not in itself

FIG. 1.—Method by which stem tip and leaves up to 2 inches in length were subjected to short photoperiod while remainder of plant was on long photoperiod. Box in position at left, detached at right.

induce nor inhibit tuber formation, the young leaves of twelve other plants were similarly trimmed; six of these were then placed on the short photoperiod bench and the remainder left on the long photoperiod bench, and used as controls.

FIG. 2.—A pair of plants approach-grafted. All leaves of plant on left subjected to short photoperiod during a part of experimental period. All parts of plant on right exposed to continuous long photoperiod.

Twenty more plants were approach-grafted as illustrated in figure 2. One leaf of one plant of each of five of the pairs was given short photoperiod. Thus with the exception of a single leaf, all the above-ground parts of both plants of each pair were subjected to long photoperiod. All the leaves of one plant of each of the remaining five grafted pairs were subjected to short photoperiod during the

first week of the treatment. As the plants grew and more foliage was produced than could be included in the box, the cover was removed from about the stem and the lid replaced at a higher level. Thus not all of the leaves were continuously exposed to short photoperiod. Such a grafted pair is shown in figure 2 just before being harvested. The boxes were put in place and removed at approximately the same hour as were the curtains on the short photoperiod bench.

In order to determine whether the artichoke can be photoperiodically induced, eighteen pots were transferred to the short photoperiod bench April 20. These were returned to the long photoperiod bench in groups of three after being exposed respectively to treatments of one, two, four, eight, twelve, and sixteen short photoperiods accompanied by correspondingly long dark periods.

Results and discussion

On June 9 all the plants were removed from the pots, the soil carefully washed away from underground portions, and tuber formation noted. In order to show tubers and stolons more clearly, most of the roots were removed before the plants were photographed. The results are shown in table 1 and in figures 3 and 4.

Of the six plants which had only the terminal buds and young expanding leaves on short photoperiod (fig. 1), only one showed the slightest indication of tuber formation, and these tubers were few in number and very small (fig. 3*B*).

Additional evidence that the stem tip is not the locus of photoperiodic perception is furnished by the six plants whose stem tips were exposed to short photoperiod and the young leaves trimmed off repeatedly. None of these produced any tubers, although the six plants similarly trimmed and exposed to short photoperiods produced as numerous and as large tubers as did the controls on short photoperiod.

Eleven plants (including five of the grafted ones) had single leaves subjected to short photoperiod while all other above-ground portions were on long photoperiod. Without exception these produced tubers (figs. 3*C*, 4*B*), although they were not so large as were those on the short photoperiod controls and did not become evident until several days after thickening stolons were first noted on short photoperiod

control plants. This evidence leads to the conclusion that when exposed to short photoperiod, changes take place in the leaves of *Helianthus tuberosus* which lead to tuber formation.

TABLE 1

TUBER FORMATION IN ARTICHOKES IN RESPONSE TO VARIOUS PHOTOPERIODIC TREATMENTS

TREATMENT	TOTAL NO. OF PLANTS	NO. OF PLANTS WITH TUBERS	NO. OF PLANTS WITHOUT TUBERS
	LONG PHOTOPERIOD CONTROLS		
On continuous long photoperiod	6	0	6
Terminal bud and leaves up to 2 inches in length on short photoperiod (fig. 1)	6	1	5
Terminal bud on short photoperiod; young leaves repeatedly trimmed	6	0	6
Short photoperiod with young leaves repeatedly trimmed from terminal bud	6	6	0
Long photoperiod with young leaves repeatedly trimmed from terminal bud	6	0	6
Long photoperiod with one leaf given short photoperiod	6	6	0
Long photoperiod grafted to plants whose upper portions were on short photoperiod (fig. 2)	5	4	1
Long photoperiod grafted to plants which had only one leaf on short photoperiod	5	3	2
Long photoperiod except for induction period of:			
One short photoperiod and one long night	3	0	3
Two short photoperiods and two long nights	3	0	3
Four short photoperiods and four long nights	3	0	3
Eight short photoperiods and eight long nights	3	0	3
Sixteen short photoperiods and sixteen long nights	3	3	0
	SHORT PHOTOPERIOD CONTROLS		
Plants on continuous short photoperiod from April 18 to June 9	6	6	0

The grafted plants (fig. 4*A*, *B*) furnish evidence which, while not conclusive, tends to confirm that part of HITCHCOCK and ZIMMERMAN'S suggestion that tuberization may be controlled by some substance with hormone-like properties. It was noted that there was a relationship between degree of development of the graft union and

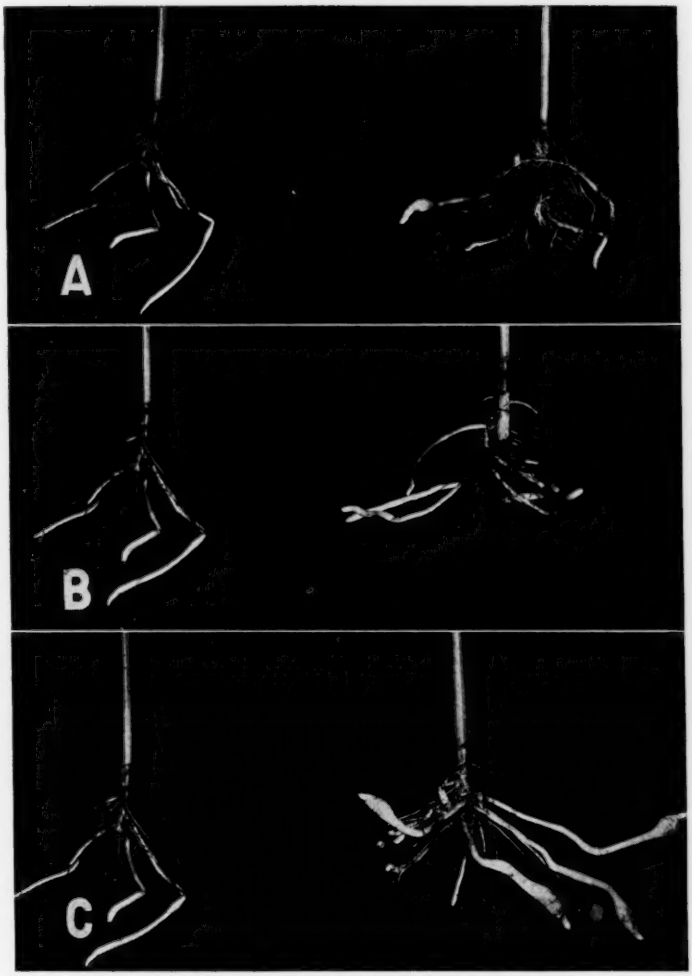

FIG. 3.—Plants at left in each case are the controls, grown on continuous long photoperiod. Plants on right received treatment as follows: *A*, grown on long photoperiod except for induction period of 16 short photoperiods beginning when plants were a little over two weeks old; *B*, terminal bud and leaves up to 2 inches in length maintained on short photoperiod; *C*, one leaf exposed to short photoperiod.

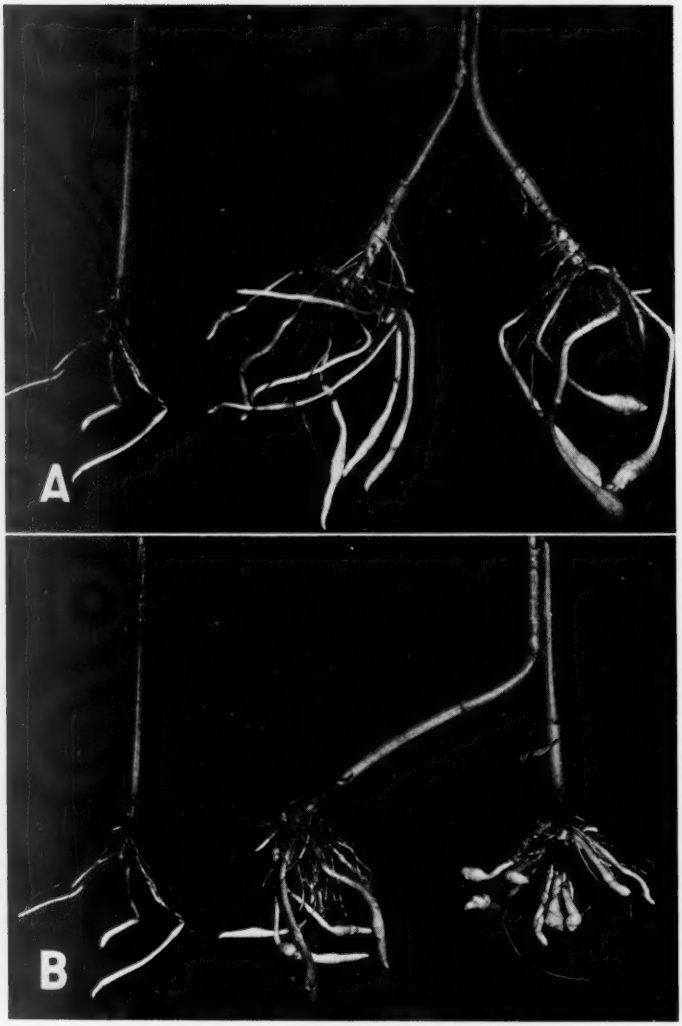

FIG. 4.—Plants at left in each case are the controls, grown on continuous long photoperiod. *A*, plant in center maintained continuously on long photoperiod but approach-grafted to plant on right, upper leaves of which were subjected to short photoperiod; *B*, plant in center maintained on long photoperiod but approach-grafted to plant on right, one leaf of which was subjected to short photoperiod.

size of the tubers of the receptor (4) plant. In those cases in which there was no evidence of transmission of a tuber-forming substance, the grafted stems fell apart when the raffia binding was removed. Whether there would have been transmission through a diffusion contact, as is true of a floral inducing substance in *Xanthium* (4), is not known.

While the tubers produced by the three plants subjected to an induction period of but sixteen short photoperiods were neither very large nor very numerous (fig. 3*A*), it seems probable that artichokes are photoperiodically induced in relation to tuber formation.

ZIMMERMAN and HITCHCOCK do not state that they included leaves in the black bags placed over the terminal buds of their plants, but they mention no measures taken to avoid that situation, nor do they indicate the dimensions of the bags. Their results can be reconciled with those presented here only by assuming that they did include leaves somewhat more mature than those exposed to short photoperiods in the small boxes in these experiments. If fairly large leaves were included in their treatment of the tip of the plant, then it may be that these leaves induced tuber formation while they were being exposed to the short photoperiod, or that they were photoperiodically induced and stimulated tuber formation even after they became too large to be included in the bags.

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A PLANT GROWTH INHIBITOR AND PLANT GROWTH INHIBITION

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(WITH ELEVEN FIGURES)

Introduction

When auxin is applied unilaterally to the top of an *Avena* coleoptile there results a negative growth curvature; that is, a curvature away from the side on which the auxin is placed. During the course of investigations of an ether extract of radish cotyledons, it was ob-

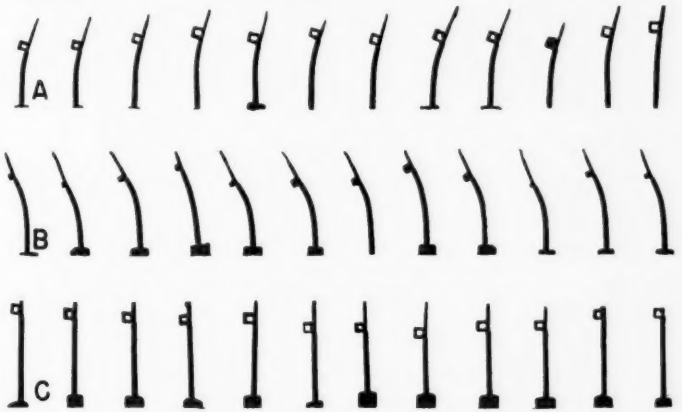

FIG. 1.—Growth curvatures of *Avena* coleoptiles: *A*, negative curvature, growth promotion by indoleacetic acid; *B*, positive curvature, growth inhibition by radish cotyledon extract; *C*, control, no curvature, blank 1.5 % agar.

served that the curvature was toward the side of application; that is, the curvature was positive (10). Examples of both positive and negative curvatures resulting from the application of growth substances are given in figure 1.

VIEHMAN (11) has found that lanolin pastes of acetic and formic acid in concentrations of from 0.0625 to 0.25 M, when applied to the top of a piece of *Helianthus* or *Lupinus* hypocotyl 1 cm. long, cause a substance to diffuse out of the bottom of the pieces which is capable

of eliciting positive *Avena* curvatures. These curvatures appear after two hours, and hence are not the same as those resulting from the inhibitor from radish which appear in less than one hour (fig. 4).

STARK (9), NIELSEN (3), and SEUBERT (7) as early as 1921 reported positive curvatures, but these were probably not caused by growth inhibiting substances as these investigators stated, but by the unequal production of auxin on the two sides of the top of the decapitated *Avena* coleoptile (14). Blank agar blocks applied to the top of such coleoptiles will cause this condition and hence positive curvatures after two and a half hours (fig. 5). GORTER (1), who first made this observation, states: "A growth retarding substance has not been found. Its appearance should cause a positive curvature of the stump within two and a half hours."

Since heretofore no substance had been found which so completely met the criteria for a true growth inhibitor, it became the object of this work to investigate the properties of this extract. In subsequent discussion reference made to growth inhibitor will refer to this substance obtained by ether extraction of radish cotyledons, variety French Breakfast, according to the method of VAN OVERBEEK (5). Inhibitor was applied to the test plants in 1.5 per cent agar blocks $2 \times 2 \times 2$ mm. in size. The Siegeshafer strain of *Avena* was used according to the technique outlined by WENT and THIMANN (14).

Investigation

BIO-ASSAY

The positive curvatures resulting from the action of the inhibitor suggested that they might be used as a quantitative biological assay for the inhibitor in the same manner that the negative *Avena* curvatures are used for the quantitative determination of growth promoting substances (14). To make certain of this, three factors had to be considered: (1) rate of curvature of the *Avena* coleoptile when treated with the inhibitor; (2) relation between degrees of positive curvature and concentration of the inhibitor; and (3) type of *Avena* test plant to use.

The rate of curvature was determined by the use of a photokymograph (6). In this device the movement of the *Avena* coleoptile is

recorded photographically every four minutes over a period of five to six hours following application of the substance being tested.

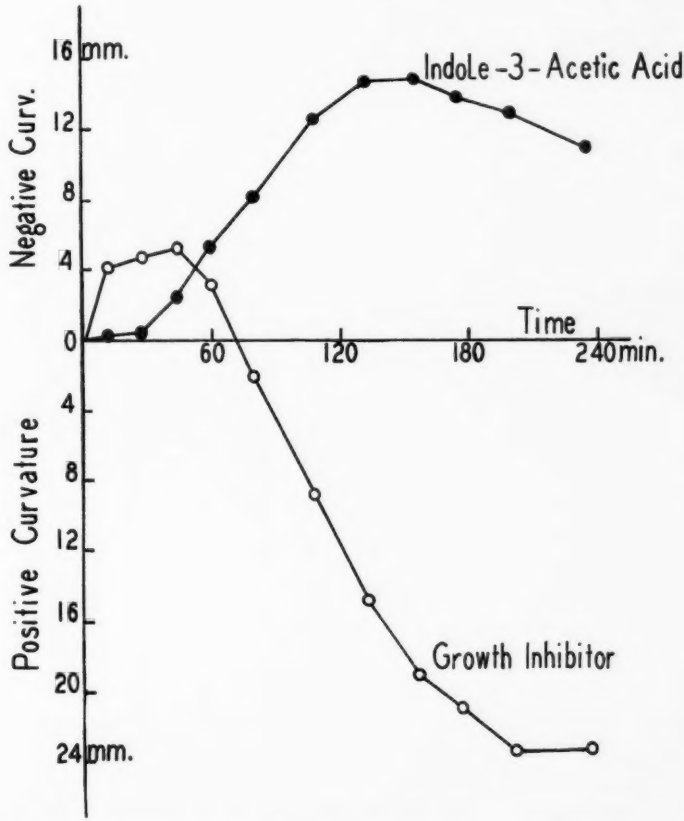

FIG. 2.—Curvature rate of once decapitated *Avena* plants upon application of: *A*, 0.05 mg./l. indoleacetic acid; *B*, growth inhibitor. Ordinate values given as mm. deviation of extended coleoptile from vertical position. Each point averages 12 test plants.

When this technique was used with the inhibitor extract it was found that after 150 minutes the maximum rate of positive curvature was still in progress, but soon thereafter decreased. The time for measuring the positive curvature was set at 150 minutes after application

of the substance being tested, instead of after 90 minutes as in the standard *Avena* test (fig. 2).

To investigate the relation between degrees of positive curvature and concentration of the inhibitor, a dilution series of inhibitor extract was prepared. Each dilution value was tested with 48 *Avena* plants. It was found that between 3° and 13° positive curvature there exists a linear proportionality to the concentration of the inhibitor (fig. 3).

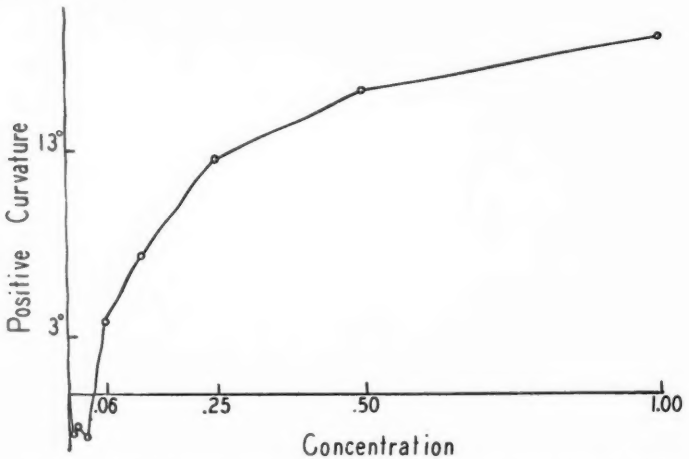

FIG. 3.—Relation between positive curvature of *Avena* plants and twofold dilutions of inhibitor substance.

By testing on the kymograph "deseeded" (**8**) once and twice decapitated *Avena* coleoptiles, and coleoptiles to which auxin had been added two hours before the test by applying it at the tip of the coleoptile in a small drop of lanolin (concentration 1:10,000), it was found that once decapitated plants showed the greatest response to inhibitor (fig. 4).

From these experiments the standard inhibitor test is defined as being the same as the standard *Avena* auxin test (**14**), except that: (1) the *Avena* coleoptile growth curvatures are positive; (2) the curvatures are measured after 150 minutes; and (3) the curvature is linearly proportional to the concentration of the inhibitor only be-

tween 3° and 13°. Although plants once decapitated show the greatest response to inhibitor, the difference between them and those

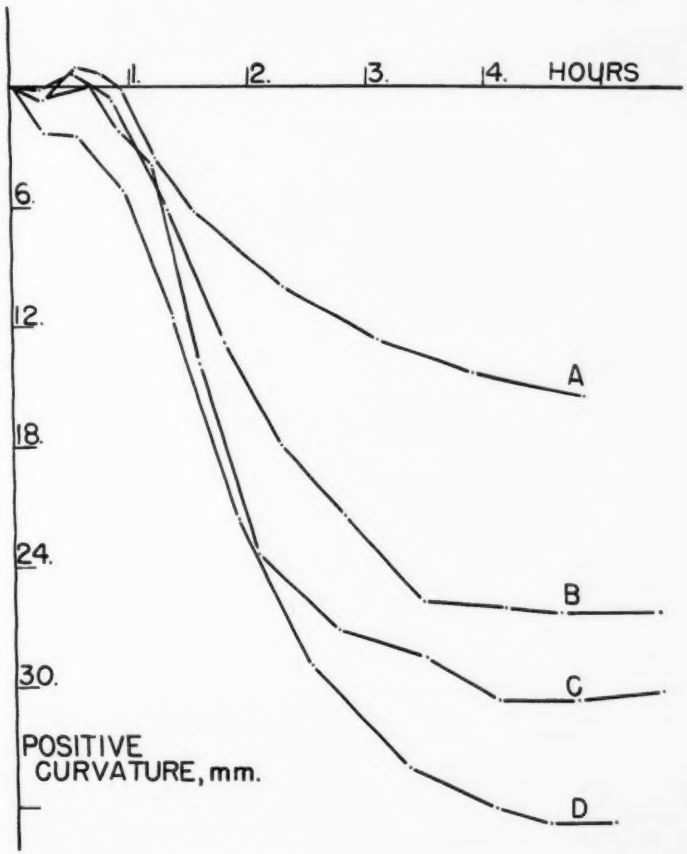

FIG. 4.—Inhibitor reaction rate of different types of *Avena* test plants: *A*, deseeded; *B*, added indoleacetic acid; *C*, twice decapitated; *D*, once decapitated. Each point averages 12 plants.

twice decapitated, after 150 minutes, is so slight that the twice decapitated coleoptiles were used as a matter of convenience except in special experiments.

SOURCES OF INHIBITOR

Using this standard method for the inhibitor test, possible sources of inhibitor were sought. It was found in the cotyledons of six varieties of radish, in the cotyledons of turnip, rutabagas, mustard, and

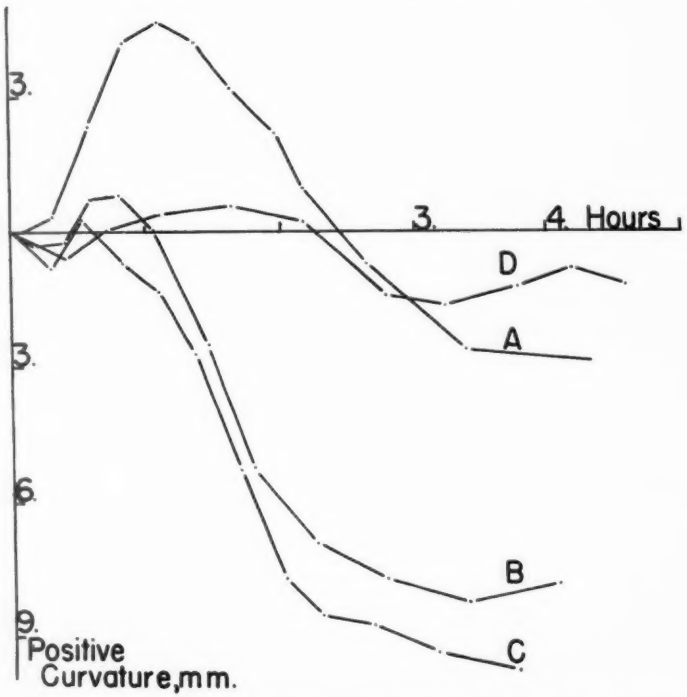

FIG. 5.—Reaction rate of twice decapitated *Avena* coleoptiles to purified inhibitor mixed with indoleacetic acid, 0.045 mg./l. *A*, inhibitor in ratio of 1:4 of indoleacetic acid; *B*, inhibitor in ratio of 4:1 of indoleacetic acid; *C*, inhibitor alone; *D*, blank agar. Each point averages 10–12 coleoptiles.

nasturtium; and it has been reported in the fruit of the pepper tree by A. J. HAAGEN-SMIT (unpublished work).

If both auxin and inhibitor were to occur simultaneously in an extract of the same material, the net curvature might be neither a measure of auxin nor of inhibitor. Only if the inhibitor were in high

enough concentration to cause a greater positive curvature than the negative curvature caused by auxin might its presence become apparent. Likewise only if the auxin were in sufficient concentration to cause a greater negative curvature than the positive curvature caused by the inhibitor would auxin become apparent. These two sets of conditions were realized by mixing indoleacetic acid (45 gamma per liter) with purified inhibitor in the ratios of 1:4 and 4:1. Figure 5 shows that the 4:1 ratio caused a 4° negative curvature, while after the same time (90 minutes) the 1:4 ratio caused a 3° positive curvature. Inhibitor with no indoleacetic acid caused a 4° positive curvature. (On the ordinate, 1 mm. deflection of the coleoptile equals approximately 1° curvature.) Thus in an ether extract of plant tissue depending on the relative amounts of auxin and inhibitor present, the *Avena* test would give curvatures that would be true measures neither of auxin nor of inhibitor.

Indirect evidence for the presence of inhibiting substances in ether extracts that mask the effect of auxin has recently been presented by GOODWIN (**2**).

From these considerations it seems likely that the occurrence of inhibitor may be rather widespread, but that it is overlooked because it occurs in too low a concentration and is accordingly masked by the auxin.

INHIBITOR IN GROWING RADISH PLANT

Radish seeds, French Breakfast, were sown in flats containing loamy soil, at intervals of three days over a period of two and a half months. During the last two weeks of this period seeds were planted every day. At the end of the two and a half months the amounts of inhibitor in the cotyledons and leaves of plants of the various ages were determined. Ether extractions of the hypocotyls showed no curvatures. Additional results are given in figure 6. A few determinations on plants of various ages were repeated at another time and found to agree with those presented here. As the plant grows the amount of inhibitor reaches a sharp maximum on the eighth day after the seeds are planted, and from then on decreases until the cotyledons finally contain no inhibitor but do contain auxin. The developing leaves, from the youngest at the tip to the fully expanded

ones, show a similar continual decrease in the amount of inhibitor with aging, but there is no indication at any time of an increase in inhibitor content as there is in the cotyledons. While figure 6 shows that the concentration of inhibitor per gram fresh weight of the leaves decreases with growth, it does not necessarily follow, because of change in size or weight of the leaf, that the amount of inhibitor per leaf decreases accordingly. A comparison of the amount of in-

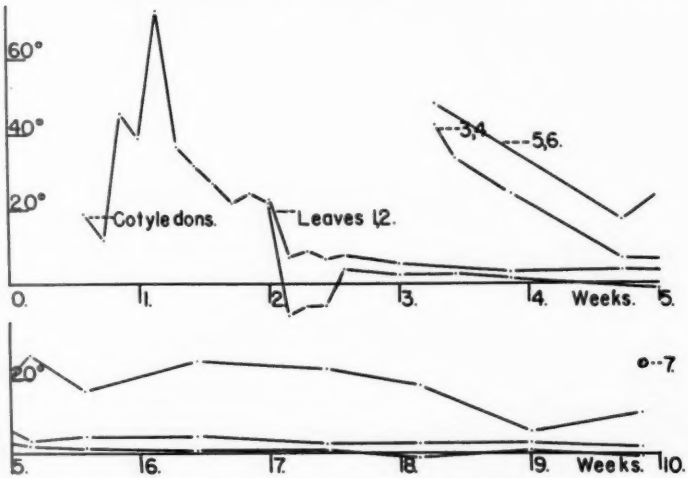

FIG. 6.—Inhibitor distribution with age in cotyledons and leaves of radish. Leaves numbered in order of appearance. Each point averages 12 test plants. Inhibitor given as degrees positive curvature per 5 gm. fresh weight material when extract is taken up in 1 cc. of 1.5 % agar.

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Chemical properties of inhibitor substance

Mr. C. E. Redemann, working with Dr. A. J. Haagen-Smit, began an investigation (unpublished) of the chemical properties of the inhibitor substance. It was found that when an active inhibitor extract was tested for its stability in acid or base, the positive curvature activity was completely lost and replaced by a strong negative curvature activity upon the alkaline hydrolysis. This made it seem likely that the substance causing the positive curvatures was a precursor of one of the auxins. It was also found that the inhibitor contained no acidic or basic groups, making it necessary to postulate for the inhibitor a compound, part of which was this auxin, but without its free carboxyl group. From the pronounced solubility in fat of the inhibitor, it seemed possible that an ester linkage between the auxin part of the molecule and the remaining portion might exist. Further work on this point is necessary.

The well established chemical properties of the inhibitor are: (1) it can be extracted from plant tissue with either di-ethyl ether or ethyl acetate; (2) it contains no acidic or basic groups; (3) it can be easily hydrolyzed to yield a growth promoting fraction; (4) certain chemical tests indicate that the growth promoting fraction is indoleacetic acid.

Effect of inhibitor on growth of Avena coleoptiles

A growth rate which is relatively less on the treated side causes a positive curvature. This relative difference in growth rate could be brought about either by an actual inhibition of growth on the treated side or by an increase of growth on the untreated side. Growth rate measurements were made as follows. The upper 15 mm. of intact *Avena* coleoptiles were marked into 3 mm. zones with India ink. Growth of these zones was then followed at 2-hour intervals by measuring the increase in distance between them. To one set of six plants purified inhibitor was applied over the tip millimeter of the coleoptile in a small drop of lanolin paste. To another set of six plants pure lanolin paste alone was applied. Throughout the course of the experiment the plants were kept in the regular *Avena* testing darkrooms, where the humidity and temperature were maintained con-

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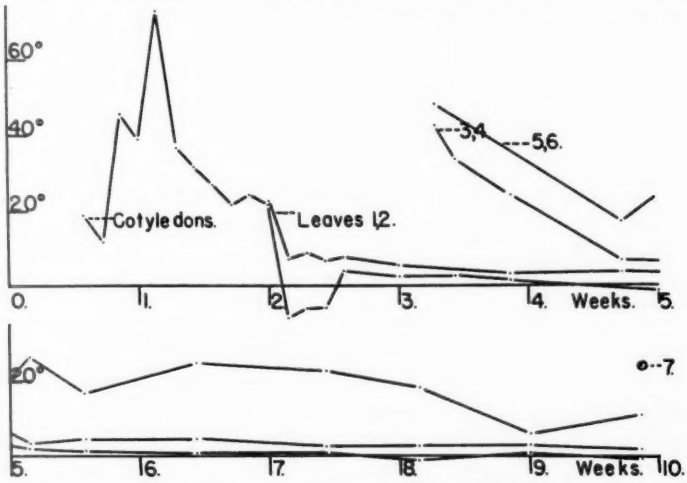

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stant at 85 per cent and 24° C. The plants were illuminated only with light transmitted through a Corning orange light filter, no. 243. The growth of these two sets of plants was measured accurately with a horizontal microscope to 0.01 mm.

The inhibitor decreased the growth rate to about one-fourth that of the controls, except in the lowest zone where it was about one-half that of the controls (fig. 7). The inhibitor, at the concentration used, stopped growth of the coleoptiles in six hours, whereas the con-

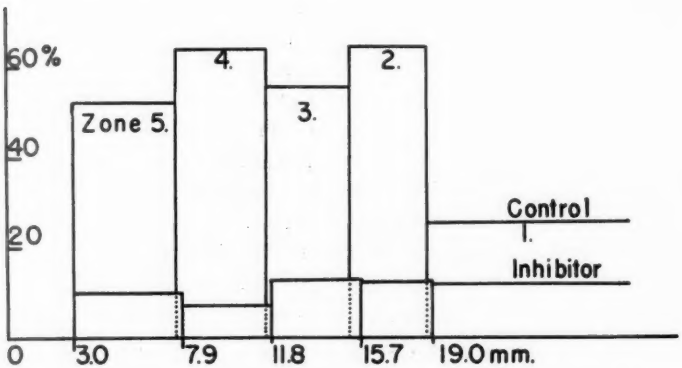

FIG. 7.—*Avena* coleoptile growth distribution when treated with inhibitor. Ordinate, percentage increase in length during 12 hours. Abscissa, coleoptile zones in mm. from tip.

trols continued to grow for six hours longer. The growth inhibition took place equally in all zones except the lowest one, where there was stimulation followed by inhibition (fig. 8). Thus it may be concluded that the relative decreased growth rate of the *Avena* coleoptile on the side treated with inhibitor is caused by an actual inhibition of growth on that side, rather than by an increase in the growth rate on the untreated side.

These results were substantiated by growth measurements on isolated sections of *Avena* coleoptiles. In these experiments 4.2 mm. sections were cut from *Avena* coleoptiles or radish hypocotyls and placed in inhibitor solutions of different concentrations. In all concentrations except the very lowest, a growth inhibition took place

when compared with growth in water alone. The very lowest inhibitor concentration showed the same amount of growth as the water controls.

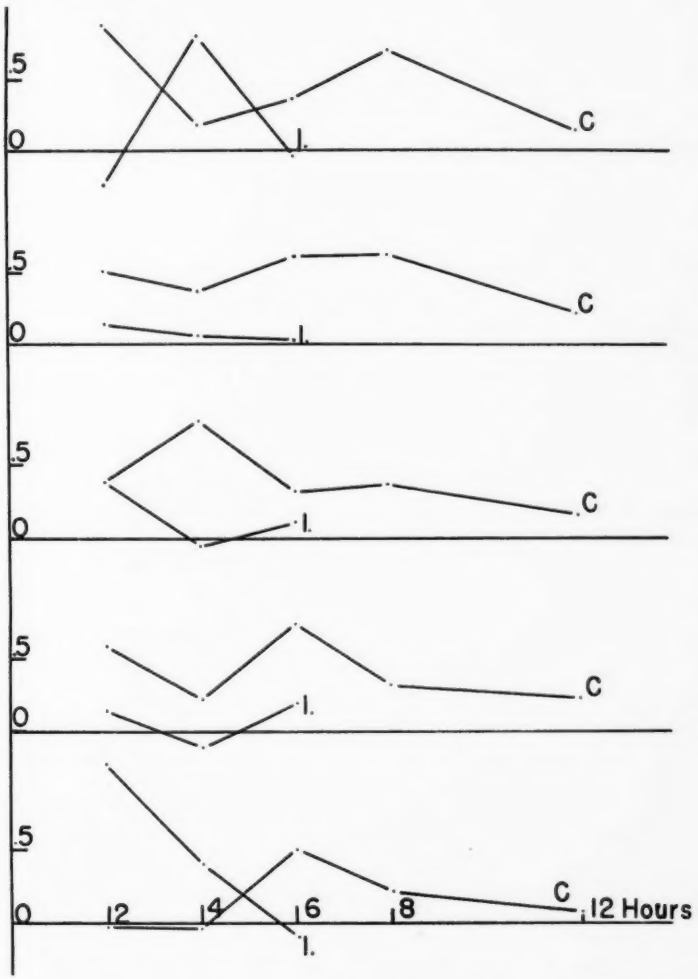

FIG. 8.—Distribution of growth rate of *Avena* coleoptiles when treated with inhibitor. Ordinate, increase in zone length in mm. *C*, control; *I*, inhibitor treated plants.

TRANSPORT POLARITY OF INHIBITOR

It has been pointed out that the inhibitor is a substance of neutral chemical character; that is, the molecule contains neither acidic nor basic groups. Accordingly it could be expected, on the basis of WENT's (13) potential gradient theory of auxin transport, that inhibitor should be transported acropetally as well as basipetally. Experiments have shown this to be the case, as equal amounts of inhibitor were found to pass through uninverted as well as inverted sections of *Avena* coleoptiles.

Similar data were found for sections of radish hypocotyls, except in one experiment where, instead of finding inhibitor in the agar blocks at the lower end of the sections being used, auxin was found. Attempts to repeat this experiment were unsuccessful and showed only inhibitor passing through the uninverted and inverted sections. Inhibitor can mask the effect of auxin, and since in this one instance auxin was found to be present in the lower blocks of the sections of radish hypocotyl which transported the inhibitor, but in no other cases, a method was devised whereby a time record could be made of any growth promoting or growth inhibiting substances which diffused from the sections. Thus subsequent masking of the first substances diffusing out of the sections was overcome. This method consisted of mounting a 4.2 mm. long section of radish hypocotyl on the shoulder of a decapitated *Avena* coleoptile. On top of the radish section was placed an agar block containing the inhibitor. The curvatures of the coleoptile were recorded photographically by means of the photokymograph.

REMOVAL OF AUXIN FROM CRUDE INHIBITOR EXTRACT

Before investigating the substances (possibly auxin) being produced by the transport of inhibitor through plant tissues, it was first necessary to be certain that there was no auxin contaminating the original inhibitor preparation.

Since it was impossible to purify the inhibitor by any known chemical means without the risk of hydrolyzing it into auxin, a physiological purification method was developed. It is well established that *Avena* coleoptiles will transport auxin only basipetally. Inhibitor substance on the other hand can be transported in both directions.

Consequently the technique was to pass inhibitor through inverted *Avena* coleoptile sections 4.2 mm. long. Because they were inverted the auxin was prevented from passing through but the inhibitor could be collected as it diffused out of the section into an agar block

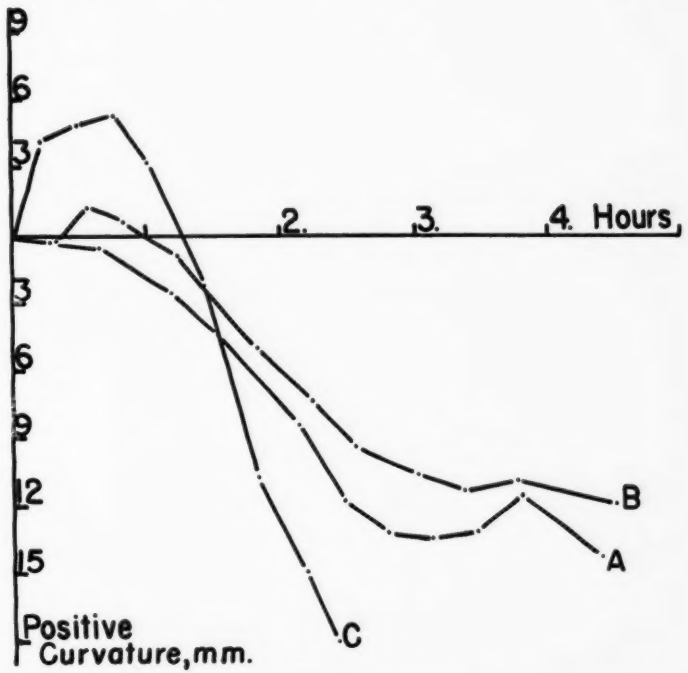

FIG. 9.—Reaction of twice decapitated *Avena* coleoptiles to purified inhibitor, two concentrations, 1 and 0.25 (*A* and *B*), and of once decapitated coleoptiles to unpurified inhibitor (*C*). Each point averages 9–12 coleoptiles.

at the other end. Generally ten to twelve hours' transport time was allowed for the preparation of such "purified" inhibitor (fig. 9). This experiment has been repeated many times with similar results.

The reaction to crude inhibitor extract; that is, a negative curvature followed by a positive one can be duplicated by using purified inhibitor and mixing indoleacetic acid with it. When this is done the same type of curvature is found as that obtained with the crude

inhibitor extract (figs. 5, 2). Apparently the negative curvatures preceding the positive curvatures (and resulting from application of the crude unpurified extract) are caused by auxin which was extracted from the cotyledons together with inhibitor.

Hydrolysis of inhibitor at cut surfaces

The one experiment which indicated that auxin diffused from inverted as well as from uninverted radish hypocotyl sections was re-

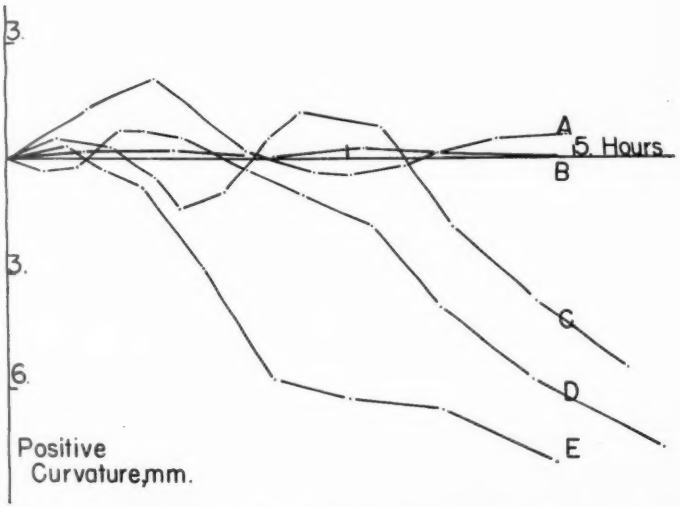

Fig. 10.—Reaction rate of twice decapitated *Avena* coleoptiles to purified inhibitor transported through inverted and uninverted 4.2 mm. sections of radish hypocotyl. Each point averages 12 coleoptiles. *A*, inverted section with blank agar; *B*, uninverted section with blank agar; *C*, inverted section with inhibitor; *D*, uninverted section with inhibitor; *E*, inhibitor applied directly, no section intervening.

peated by the kymograph technique, using auxin-free inhibitor. Negative curvatures were initiated even when the inhibitor was passed through the inverted sections which are known not to transport auxin. These initial negative curvatures soon were masked with the transport of more inhibitor and became positive (fig. 10). This experiment was repeated with sections of *Avena* coleoptiles with essentially the same results (fig. 11). Both experiments have been

repeated several times with similar results. From these experiments it appears that here, as well as in the one exceptional experiment, a small amount of inhibitor was hydrolyzed into auxin at the cut surfaces of the transporting sections and test plants.

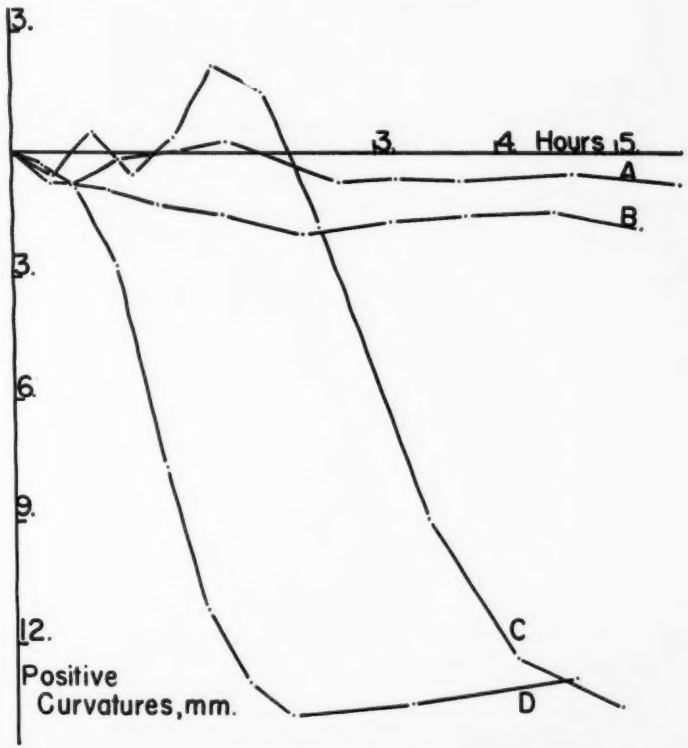

FIG. 11.—Reaction rate of twice decapitated *Avena* coleoptiles to purified inhibitor transported through inverted 4.2 mm. sections of *Avena* coleoptiles. *A*, *B*, average of 6 coleoptiles; *C*, *D*, average of 12 coleoptiles. *A*, blank agar applied directly, no section intervening; *B*, indoleacetic acid, 0.5 mg./l., inverted section; *C*, purified inhibitor, inverted section; *D*, purified inhibitor applied directly, no section intervening.

From this and similar experiments, the velocity of the transport of inhibitor either acropetally or basipetally through *Avena* coleoptile sections was found to be 11 mm. per hour, or the same as the transport velocity of auxin.

PHYSIOLOGICAL ROLE OF INHIBITOR

In many different tests of physiological activity, such as the pea test for growth substances, root growth inhibition test, seed germination, etc., inhibitor was found to act like auxin because during the course of the test it actually was hydrolyzed into auxin. In the case of bud inhibition, where the inhibitor was used in anhydrous lanolin paste and accordingly could not hydrolyze easily, it was unable to act like auxin and did not inhibit the outgrowth of buds. This experiment, however, is inconclusive since the inhibitor paste caused injury, so that no active substances could reach the lower parts of the branch.

Whether inhibitor acts as a growth inhibitor in the radish plant itself is open to question.

It has been shown by VAN OVERBEEK (**4**) that an auxin diffuses from the cut surface of radish cotyledon petioles. No indication is given, however, as to whether this auxin is indoleacetic acid, auxin-a, or auxin-b. As one of the best means of distinguishing between the auxins is by their molecular weights, molecular weight determinations by the diffusion technique (**12**) were made for: (1) the auxin coming from the cut surface of radish cotyledon petioles; (2) the purified inhibitor; and (3) indoleacetic acid (control). The molecular weights of the auxin coming from the cotyledons and of the inhibitor itself were determined in three experiments. In the case of the auxin, twelve *Avena* test plants were used to determine the relative concentration in each of the agar plates. In the case of the inhibitor, thirty-six (or in one instance forty-eight) test plants were used. Values determined for the molecular weight of the auxin coming from the cotyledons were 144, 104, and 125. The values for the molecular weight of the inhibitor were 142, 117, and 83. The value for the control, indoleacetic acid, known molecular weight of 175, was 159.

The diffusion method of determining molecular weights is not so useful for establishing exact molecular weights as it is for indicating substances of known molecular weight. In this case the auxin diffusing from the cotyledons is apparently neither auxin-a nor auxin-b (molecular weights 328 and 310); it may be indoleacetic acid. A

chemical study of the auxin obtained by hydrolysis of inhibitor likewise indicates that it may be indoleacetic acid. It is interesting to speculate as to the likelihood of the diffusing auxin originating by hydrolysis of the inhibitor in the cotyledons.

Summary

1. There is an ether-extractable substance in the cotyledons and leaves of radish plants that is capable of causing growth inhibition and positive *Avena* coleoptile curvatures.

2. It is possible to analyze for this substance on a quantitative basis.

3. This substance occurs in other plants as well as radish.

4. With growth of radish leaves their inhibitor content decreases and is finally replaced by auxin.

5. This inhibiting substance has no acidic or basic groups and can readily be hydrolyzed to form a growth promoting substance.

6. Inhibitor has no polarity of movement in the radish or *Avena* plant.

7. Auxin contaminating the crude inhibitor extract may be removed by use of the "inverse transport" purification method.

8. Inhibitor may be hydrolyzed at cut plant surfaces to form auxin.

9. Inhibitor is transported through *Avena* coleoptiles at approximately 11 mm. per hour, or at the same rate as auxin.

10. In many tests for physiological activity, inhibitor behaves like auxin.

11. The auxin diffusing from radish cotyledons is possibly indoleacetic acid.

This work was done at the California Institute of Technology, Pasadena, California.

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EFFECTS OF SOIL TEMPERATURE, pH, AND NITROGEN NUTRITION ON THE DEVELOPMENT OF POA PRATENSIS[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 503

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Introduction

Few studies have been made in which plants were grown under the combined influence of controlled nitrogen nutrition, temperature and reaction of the culture medium. In the present series of experiments bluegrass, *Poa pratensis* L., was chosen as a representative plant, sometimes grown in the field under semi-controlled nutrient conditions and differing widely in development with seasonal variation in temperature and soil reaction. The experimental plants were grown with a controlled supply of nitrogen as nitrate or ammonium, under controlled soil temperatures and reaction of the culture media. Measurements of leaf and root growth were taken to express development under the environmental conditions maintained and as a measure of the effects of periodic clipping.

The influence of the reaction of the culture medium on absorption and assimilation of nitrate and ammonium ions has been studied by many workers. NIGHTINGALE (11) has critically reviewed the literature dealing with nitrogen nutrition of green plants, and PARDO (14) reviewed the status of ammonium nutrition. The relation of temperature to nitrogen nutrition has been studied by HARRISON (5) on *Poa pratensis* and by NIGHTINGALE (9, 10, 12, 13) on several plants, including tomatoes, apple trees, and peach trees. Numerous field studies have been conducted on bluegrass, and several greenhouse experiments, notably those by HARRISON (4, 5), AHLGREN (1), GRABER (2), and GRABER and REAM (3), have been concerned with clipping effects.

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Experimental data and results

Unclipped series

Eleven-weeks experiment.—To determine the effects of nitrate and ammonium nutrition at varying pH and soil temperatures on the development of unclipped *Poa pratensis* plants, an experiment was set up using the temperature tank apparatus described by Link (6).

Plants obtained by vegetative propagation from a single plant were grown in 2-gallon crocks filled with acid-washed quartz sand. Two nutrient solutions, one containing nitrogen in the form of nitrate and the second in the form of ammonium, were made up in the usual manner, according to the concentrations outlined in table 1.

TABLE 1

Composition of nutrient solutions (partial volume molecular concentrations of salts used)

Solution	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	$CaCl_2$	$(NH_4)_2SO_4$
Nitrate	0.00211	0.00286	0.00710	.....	.....
Ammonium	0.00211	.....	0.00710	0.00286	0.00286

These concentrations are taken from those of Naftel (8). Solutions of three pH values, 4.5, 5.5, and 6.5, were made from each of these stock solutions by the addition of N/10 NaOH or N/10 H_2SO_4 until the desired pH was obtained, colorimetrically measured. The experiment was run in quadruplicate, each of the six nutrient conditions being maintained at three soil temperatures, 15°, 25°, and 35° C. The plants were grown from March 3 to May 10, 1936, under greenhouse conditions and with a relatively large amount of sunshine.

Nutrient solution was added to each culture in 400 cc. amounts twice daily. Regulation of the nutrient medium to the desired pH was accomplished by flushing each culture with distilled water before each nutrient addition. Sufficient water at the desired pH was added to bring the cultures to the proper reaction as determined by testing the leachate colorimetrically. In this way the reaction of each culture was regulated twice daily and the change in pH of the nutrient

solution as indicated in table 2 was manifested over a period of ten hours. It will be seen from the table that plants supplied with nitrate nitrogen at three pH values did not appreciably change the

TABLE 2

WEEKLY PH READINGS ON CULTURE SOLUTIONS CONTAINING PLANTS GROWN FOR 11 WEEKS IN SAND AT THREE SOIL TEMPERATURES AND NITRATE AND AMMONIUM NUTRITION AT VARIED PH

No. of weeks	15° C.			25° C.			35° C.		
	4.5	5.5	6.5	4.5	5.5	6.5	4.5	5.5	6.5
	Nitrate								
1	5.0	5.4	6.4	5.0	5.5	6.5	5.0	5.7	6.5
2	5.0	5.6	6.4	5.1	5.7	6.4	5.1	5.8	6.4
3	5.1	5.7	6.6	5.1	5.6	6.2	5.2	5.8	6.5
4	5.0	5.8	6.6	5.0	5.6	6.2	5.1	5.6	6.4
5	5.1	5.7	6.5	5.0	5.4	6.0	5.1	5.5	6.2
6	5.0	5.5	6.6	4.0	5.3	6.5	5.0	5.6	5.4
7	5.0	5.5	6.5	5.0	5.4	6.3	5.0	5.6	6.2
8	4.8	5.6	6.4	4.6	5.4	6.5	5.0	5.6	6.3
9	5.0	5.5	5.8	5.0	5.0	6.2	5.0	5.6	6.2
10	5.8	5.4	6.2	5.0	5.6	6.3	5.0	5.2	6.3
	Ammonium								
1	5.0	5.4	6.4	5.4	5.5	6.5	5.2	5.5	6.5
2	5.0	5.6	6.4	5.2	5.8	6.6	5.3	5.8	6.5
3	5.4	5.6	6.5	5.3	5.5	6.1	5.3	5.6	6.0
4	4.8	5.6	6.6	5.3	5.3	5.2	5.2	5.5	5.2
5	5.1	5.5	6.5	5.1	5.3	5.0	5.0	5.2	4.6
6	5.0	5.5	6.5	4.8	5.1	5.6	5.0	5.3	5.0
7	4.8	5.5	6.3	4.6	5.4	5.0	5.0	5.3	4.9
8	5.0	5.5	5.8	4.6	5.0	5.3	4.6	5.2	5.0
9	5.0	5.5	5.4	4.7	5.2	5.3	4.6	5.4	5.0
10	5.0	5.2	5.0	5.0	5.0	5.6	5.0	5.0	5.4

reaction of the culture medium over this half-day period, with the exception of the pH 4.5 solution. In general, both nitrate and ammonium solutions applied at pH 4.5 changed rapidly to pH 5.0 and remained fairly constant at that value. Plants supplied with ammonium at pH 6.5 brought about a decided increase in hydrogen ion concentration of the medium, especially at the higher temperatures.

Some increase was noted in the case of solutions supplied at a pH of 5.5.

Measurements were taken for each culture of the length and number of leaves, green and dry weights of roots and leaves, and the number of rhizomes produced. These data are given in tables 3 and 4. Table 3 shows the decided superiority of growth, as evi-

TABLE 3

LEAF AND RHIZOME DEVELOPMENT OF PLANTS GROWN FOR 11 WEEKS IN SAND AT THREE SOIL TEMPERATURES AND NITRATE AND AMMONIUM NUTRITION AT VARIED pH. AVERAGES BASED UPON FOUR PLANTS

TEMPERATURE AND pH	PERCENTAGE INCREASE LEAF LENGTH		PERCENTAGE INCREASE LEAF NUMBER		PERCENTAGE LINEAR INCREMENT		NO. OF RHIZOMES	
	NO_3	NH_4	NO_3	NH_4	NO_3	NH_4	NO_3	NH_4
15° C.								
4.5	198	31	955	85	3048	145	16.5	3.2
5.5	194	54	942	229	2514	408	18.0	2.8
6.5	191	144	1046	524	2847	1434	32.0	7.5
25° C.								
4.5	173	75	600	35	1842	265	28.0	1.0
5.5	233	115	401	148	1585	442	33.0	3.3
6.5	182	192	620	314	1799	1015	38.0	10.7
35° C.								
4.5	140	23	263	0	761	4	28.5	0
5.5	133	55	226	53	801	141	24.3	1.3
6.5	148	66	170	80	589	209	14.5	2.0

denced by increase in leaf length and number of leaves and rhizomes produced, of the nitrate-supplied plants as compared with ammonium-supplied plants under corresponding temperature and pH conditions. The data for linear increment (a combined measure of leaf length and number) show that nitrate-supplied plants produced almost twice as much growth as plants with ammonium nutrition under pH conditions most favorable for ammonium nutrition. Differences in reaction had no appreciable effect on leaf development of nitrate plants at 15° and 25° C. At 35° C., however, a slight increase in the number of leaves and rhizomes of pH 4.5 plants over the other plants at that temperature was noted. Ammonium plants showed

the least increase in length and number of leaves at pH 4.5 and the best development at pH 6.5 under all temperature conditions.

Temperature effects on leaf development were also evident. In all cases the least development occurred under the highest temperature conditions. The greatest number of leaves was produced at 15° C., whereas increase in leaf length was almost equal at 15° and

TABLE 4

GREEN AND DRY WEIGHTS OF PLANTS GROWN FOR 11 WEEKS IN SAND AT THREE SOIL TEMPERATURES AND NITRATE AND AMMONIUM NUTRITION SUPPLIED AT VARIED PH. AVERAGE WEIGHT (IN GRAMS) BASED UPON FOUR PLANTS

Temperature and pH	Leaves				Roots			
	Green weight		Dry weight		Green weight		Dry weight	
	NO_3	NH_4	NO_3	NH_4	NO_3	NH_4	NO_3	NH_4
15° C.								
4.5	17.0	1.1	3.40	0.26	12.6	2.4	4.06	0.85
5.5	20.5	2.1	3.48	0.58	14.4	3.1	3.96	1.07
6.5	20.1	8.1	3.59	1.71	17.0	7.5	4.50	2.65
25° C.								
4.5	10.7	1.0	2.58	0.24	14.0	2.3	5.62	0.91
5.5	13.8	2.7	3.27	0.88	12.8	3.2	4.25	1.10
6.5	13.1	7.5	3.13	1.77	13.5	7.5	4.78	2.00
35° C.								
4.5	5.0	0.8	1.62	0.14	7.0	1.6	3.31	0.44
5.5	5.0	1.2	1.56	0.27	7.0	1.7	2.91	0.65
6.5	3.5	1.9	1.13	0.43	5.0	2.5	2.21	0.87

25° C. If linear increment is used as a growth index, it is seen that greatest growth, in nitrate plants especially, occurred at the lowest temperature, with decided reductions at higher temperatures. No decided tendencies were noted in rhizome production with respect to temperature, although the maximum number in both nitrate and ammonium plants was produced at the median temperature.

Table 4 shows another index of growth under the conditions of the experiment in the form of green and dry weights of the leaves and roots. The decided superiority in growth of nitrate-supplied over ammonium-supplied plants was evident in all cases. Differ-

ences in weight of nitrate plants owing to pH effects were found only at the highest temperature, at which pH 4.5 plants were superior. Ammonium plants produced the greatest amounts of leaves and roots at pH 6.5. With both nitrate and ammonium nutrition, the root and leaf weights of plants grown at 15° and 25° C. were decidedly superior to corresponding plants grown at 35° C.

Examination of the root systems of the nitrate and ammonium plants showed a general similarity when grown at 15° and 25° C. High temperature caused the production of a root system only half the size produced at lower temperatures.

THREE AND SIX WEEKS EXPERIMENTS.—Two additional series were grown in order to furnish information concerning the early development of plants grown under the conditions of the previous experiment. Two plants were grown in each condition of nutrient supply, temperature, and pH. The procedure described in the previous experiment was followed with the exception that duplicate plants were placed in the same container. For the length of time the experiment was conducted, the amount of nutrient added was sufficient for both plants. The two series were planted on April 26, 1936, and nutrient treatments started on May 4 and continued to May 26 and June 12 respectively for the three and six weeks series. The weather conditions during this period were perhaps a little more favorable for plant growth than during the period of the previous eleven weeks experiment, as longer days and clearer weather prevailed.

Determinations of the reaction of the culture solutions were made at weekly intervals and showed essentially the same general trend in pH that prevailed in the previous experiment. At the end of the sixth week of growth, plants supplied with ammonium at pH 6.5 were active enough to convert the culture solution to pH 5.0. Some increase in acidity of the pH 6.5 solution containing nitrate was noted, but in general the nitrate-supplied plants effected little change in the acidity of the culture solutions.

The development of the plants in these two series was followed in the same manner as in the other experiment, and the results for leaf and rhizome production are shown in tables 5 and 6. The type of response to temperature and nutrient supply was similar in these

shorter series to that shown in the eleven weeks experiment. At the end of three weeks, plants supplied with nitrate at pH 6.5 had superior leaf development except under high temperature conditions, but observations at the end of six weeks showed little difference in pH effect. Ammonium plants showed the best leaf and rhizome development at pH 6.5 at all temperatures, and in all cases the development of nitrate plants was superior to that of ammonium plants under corresponding conditions. Rhizome production was restricted in the ammonium-supplied plants and no significant differences in number of rhizomes as related to pH could be noted in the nitrate plants.

TABLE 5

LEAF AND RHIZOME DEVELOPMENT OF PLANTS GROWN FOR THREE WEEKS IN SAND AT THREE SOIL TEMPERATURES AND NITRATE AND AMMONIUM NUTRITION SUPPLIED AT VARIED PH. AVERAGES BASED UPON TWO PLANTS

Temperature and pH	Percentage increase leaf length		Percentage increase leaf number		Percentage linear increment		No. of rhizomes	
	NO_3	NH_4	NO_3	NH_4	NO_3	NH_4	NO_3	NH_4
15° C.								
4.5	35	0	73	16	131	16	2.5	0
5.5	40	8	99	13	179	12	3.0	0
6.5	56	18	112	24	233	47	4.0	0
25° C.								
4.5	23	0	90	0	134	0	4.0	0
5.5	33	17	94	11	157	30	2.5	1
6.5	65	16	107	27	244	50	0	0
35° C.								
4.5	20	0	71	0	105	0	0	0
5.5	0	0	64	0	64	0	0.5	0
6.5	7	0	34	6	46	0	0	0

Green and dry weights of both three and six weeks plants were taken and showed generally the same relations as seen in the eleven weeks experiment. Differences in green and dry weights at the various temperatures were small, especially at 35° C., but a tendency prevailed for maximum weight at pH 6.5 in both nitrate and ammonium plants.

The character of the root systems varied with temperature treatment and with the form of nitrogen nutrition. At the lowest temperature the roots were large in diameter, white, and very succulent, whereas at 35° C. the roots were small, light brown, and very finely branched, forming a densely tufted system. The zone of meristematic development and elongation was very short in roots grown

TABLE 6

LEAF AND RHIZOME DEVELOPMENT OF PLANTS GROWN FOR SIX WEEKS IN SAND AT THREE SOIL TEMPERATURES AND NITRATE AND AMMONIUM NUTRITION SUPPLIED AT VARIED pH. AVERAGES BASED UPON TWO PLANTS

TEMPERATURE AND pH	PERCENTAGE INCREASE LEAF LENGTH		PERCENTAGE INCREASE LEAF NUMBER		PERCENTAGE LINEAR INCREMENT		NO. OF RHIZOMES	
	NO_3	NH_4	NO_3	NH_4	NO_3	NH_4	NO_3	NH_4
15° C.								
4.5	90	0	227	118	530	118	18.0	1.5
5.5	110	18	304	135	748	179	12.0	2.0
6.5	64	50	547	373	937	603	17.5	9.5
25° C.								
4.5	100	8	500	71	1049	81	19.0	1.0
5.5	150	25	385	47	1118	88	18.0	1.0
6.5	136	41	339	272	944	421	18.5	6.0
35° C.								
4.5	40	8	219	0	343	8	11.0	0
5.5	30	8	332	8	467	12	5.5	0
6.5	21	30	109	39	121	81	10.0	0

at high temperatures, as shown by the presence of laterals almost to the tips of the main roots. Limited data on root tip length indicate that the greatest amount of root elongation took place at the lowest temperature.

Little difference could be noted in roots of nitrate plants with respect to pH value of the culture. Ammonium plants, however, at pH values of 4.5 and 5.5 showed a scant development of lateral roots as compared with plants grown at pH 6.5. At all temperatures the secondary laterals on ammonium plants were very short or stunted and discolored for the proximal 5–8 cm. of the newly

developed crown roots. This reduced condition of the laterals was not noted in nitrate plants.

The leaves of plants grown in this series and the former series show marked differences in degree of succulence as influenced by temperature and pH treatment. The leaves of both nitrate and ammonium plants were more succulent at low temperatures than at high ones. Nitrate plants showed little difference with respect to pH value of the culture at any one temperature, but in the case of ammonium-supplied plants, those grown at pH 6.5 were more succulent than plants grown at pH 4.5 or 5.5. This difference in succulence was more marked at 15° than at 35° C.

Clipped series

To furnish information more directly applicable to turf production, an additional experiment was initiated involving the clipping of *Poa pratensis* plants under nitrate and ammonium nutrition at a uniform pH and soil temperature. The temperature tank apparatus was again used to maintain the soil temperatures at 25° C., and both nitrate and ammonium nutrition were supplied as in the previous experiments at pH 6.5. Plants were divided into three lots: clipped at 1 inch weekly, at 2 inches weekly, and control. Ten plants were used for each combination of nutrition and clipping height, and the plants were grown under greenhouse conditions from May 30 to September 14, 1936.

Clipping treatments were started on June 26 and made weekly thereafter, and the number of leaves clipped and the dry weights of the clippings were secured. Weekly counts were also taken of the total number of leaves on all clipped and control plants. Table 7 shows the percentage increase in number of green leaves for each week, based on the original number of leaves. The effects of clipping are shown in the decided reduction in the leaf production of the 1 inch series and the slight reduction in the 2 inch series, compared with the controls. Comparison of the check plants in nitrate and ammonium nutrition shows a more rapid production of leaves in ammonium nutrition throughout the experimental period, except during the first two weeks. Nitrate plants clipped at 2 inches produced more leaves than ammonium plants during the first six weeks

of the experiment, but nitrate plants clipped at 1 inch had in general a smaller production of leaves than corresponding ammonium plants throughout the experiment.

Total dry weights of the weekly clippings are shown in table 8. Nitrate-supplied cultures showed consistently higher yields than ammonium plants, although in most cases this difference was not significant. With the exception of a brief initial period in which insect infestation reduced the number of leaves, there was a consistent increase in yield of clippings over the experimental period in

TABLE 7

PERCENTAGE CUMULATIVE INCREASE OF NUMBER OF LEAVES PRODUCED BY PLANTS GROWN IN SAND WITH NITRATE AND AMMONIUM NUTRITION UNDER CLIPPED AND UNCLIPPED CONDITIONS

Treatment	July 11	July 18	July 25	Aug. 1	Aug. 8	Aug. 15	Aug. 24	Sept. 1	Sept. 8	Sept. 14
Nitrate										
Check	10.1	33.5	71.6	92.5	129.7	150.9	181.3	204.3	219.0	243.0
1 inch	6.1	18.1	38.6	55.7	70.4	84.5	92.8	101.2	100.3	99.5
2 inches	5.0	31.9	61.3	83.4	112.4	135.8	162.9	180.0	192.4	159.6
Ammonium										
Check	7.4	27.5	74.2	104.7	136.9	162.1	205.2	260.1	285.0	307.2
1 inch	1.9	16.5	37.9	55.4	72.3	95.2	124.2	148.8	157.4	192.7
2 inches	.....	19.0	51.1	74.1	104.6	126.8	164.5	186.5	208.8	200.9

both nitrate and ammonium cultures, particularly in the 2 inch clipping series. Comparison of the yields obtained from the 1 inch and 2 inch series shows the 2 inch nitrate plants to have a greater yield than plants cut at 1 inch. At the end of the experiment, nitrate plants cut at 2 inches were producing three times as much as plants kept at a 1 inch height, and the total amount of clippings obtained throughout the experiment from the 2 inch series was double that for the 1 inch plants. Ammonium-supplied plants, on the contrary, produced a greater amount of clippings during the first four weeks of the experiment when kept at a 1 inch height; and during the last six weeks those plants clipped at 2 inches produced a slightly larger yield than 1 inch plants. Total weight of clippings from the 2 inch ammonium plants was only slightly larger than that obtained from the 1 inch plants.

Comparison of root and rhizome production in nitrate and ammonium-supplied plants showed in general the same relationships as in the previous experiments. Nitrate plants produced a greater number of crown roots than ammonium plants although the average length of roots was greater in the case of ammonium plants. The check plants showed the greatest number of roots and rhizomes under both forms of nutrition, and the 1 inch clipping series showed the poorest developed root systems. A greater number of rhizomes was produced under nitrate nutrition than with ammonium nitrogen.

TABLE 8

DRY WEIGHT (GRAMS) OF CLIPPINGS OBTAINED FROM PLANTS GROWN IN SAND WITH NITRATE AND AMMONIUM NUTRITION AND CLIPPED AT 1 INCH AND 2 INCH HEIGHTS AT WEEKLY INTERVALS

TREATMENT	JULY 3	JULY 11	JULY 18	JULY 25	AUG. 1	AUG. 8	AUG. 15	AUG. 24	SEPT. 1	SEPT. 8	SEPT. 14	TOTAL
Nitrate												
1 inch	0.362	0.310	0.183	0.283	0.392	0.371	0.504	0.619	0.660	0.463	0.492	4.64
2 inches	.416	.327	.134	.377	.737	.800	.950	1.499	1.870	1.218	1.002	9.33
Ammonium												
1 inch	.335	.260	.143	.284	.305	.328	.303	.429	.516	.401	.375	3.70
2 inches	0.243	0.229	0.116	0.240	0.369	0.348	0.401	0.583	1.096	0.697	0.594	4.92

Discussion

TEMPERATURE RELATIONS

If the factor of temperature is considered apart from the complex of environmental conditions under which the plants were grown, certain relations of development with temperature differences may be established. The character of the top growth varied considerably within the range of soil temperature used. Low temperatures appeared to be the most favorable both for the production of new leaves and growth in length of leaves. The type of growth resulting at 15° C. was bushy, with long, succulent leaves and numerous new leaves produced. Combined measurements of leaf length and number of leaves or linear increment show the optimum growth temperature to be about 15° C. At soil temperatures of 35° C. the plants were short and rigid, many of the leaves remaining erect

when allowed to grow uncut for three months. Bud initiation and leaf production were limited under the high temperature conditions.

Although chemical studies were not made upon the plants grown at various temperatures, the results can be interpreted partially upon the carbohydrate-nitrogen relationships, as has been done by NIGHTINGALE and others (**9, 12, 13**). Bluegrass plants in the present experiments, as well as those of HARRISON (**5**), showed the greatest amount of root and top growth at low temperatures. In both nitrate and ammonium nutrition, absorption of nitrogen took place readily enough at low temperatures so that assimilation and synthesis of protein materials resulted in pronounced increases in leaves and leaf length. High temperatures undoubtedly favored more rapid absorption of nitrogen, as indicated by pH changes in the culture solutions, but assimilation probably did not occur so readily because of the decreased carbohydrates resulting from an unfavorable photosynthesis/respiration balance. As TIEDJENS and ROBBINS (**19**) point out, nitrate and ammonium ions may accumulate in plants which stop active growth so that apparently the bluegrass plants grown at high temperatures were limited more by carbohydrates than by nitrogen. These implications are further borne out by the yields of top growth produced under the different temperatures and by the number of rhizomes put out by the plants. Equal weights of top growth were produced by plants grown at 15° and 25° C., whereas under soil temperatures of 35° C. the lowest weight of tops and smallest number of rhizomes were produced. Many field and greenhouse experiments with bluegrass and other plants have shown the production of rhizomes to be associated with carbohydrate storage. Under the high temperature conditions of the present experiment, carbohydrates were probably utilized in respiration faster than they were being produced, and consequently few reserves in the form of rhizomes could be established.

The character of the root systems produced was also influenced strongly by temperature. At the lowest temperature the roots of bluegrass were large in diameter, succulent, white, and with few scattered laterals, whereas at 35° C. the roots were small, light brown, and very finely branched into a dense tufted system. These observations agree with those of NIGHTINGALE (**10**) on apple and

peach roots. The character of the root systems seemed to be influenced by the rate of growth and maturation as evidenced by the root tip lengths. High temperatures favored rapid maturation of roots, with consequent production of laterals almost to the extreme tips of the main roots. At low temperatures more rapid elongation occurred before maturation, causing relatively long tips. The entire root systems at 15° and 25° C. were almost twice as deep as those produced at 35° C. Total root growth as measured by dry weight was equal at 15° and 25° C. and the lowest dry weights were produced at the highest temperature. HARRISON (**5**), working with bluegrass at temperatures of 60°, 80°, and 100° F., found that no new roots were produced with nitrate nitrogen at any of the root temperatures used over a period of two months. Essentially the same results were obtained in the present investigation, as the nitrate plants produced very few new crown roots at any temperature, whereas with ammonium nutrition many new roots were present.

pH RELATIONS

Under controlled nutrient conditions with nitrogen supplied in the form of nitrate, the plants differed in top and root development with varied pH, depending in part upon their age. Plants of the three weeks series showed a slight tendency for superior development at pH 6.5 at temperatures of 15° and 25°, but at 35° C. the pH 6.5 plants were decidedly inferior in linear increment to plants grown at other pH values. After six and eleven weeks growth, plants grown under similar conditions showed no significant differences with pH at the lower temperatures, but at 35° C. plants grown at pH 6.5 were slightly inferior to pH 4.5 and 5.5 plants. Under the conditions of these experiments the range of pH used apparently had little effect upon absorption and assimilation of nitrate nitrogen except at a soil temperature of 35° C. With high temperatures resulting in more active respiration, the carbohydrate reserves in these plants subjected to high temperatures were at a minimum and assimilation of the absorbed nitrogen was governed more closely by pH. Only under these conditions do the present experiments agree with those of other investigators, as for example, SPRAGUE (**16**), who found that bluegrass supplied with nitrate and a small amount of

ammonium sulphate produced less top growth at pH 6.5 than at pH 4.5. Root development and rhizome production in nitrate cultures were influenced by pH in the same manner as top development. Weight of roots varied little with differences in pH except at 35° C., under which condition the pH 4.5 plants produced a greater weight of roots.

Plants supplied with nitrogen in the form of ammonium at three pH values showed best development in tops, roots, and rhizomes at pH 6.5, throughout periods of three, six, and eleven weeks growth. The pH range chosen for these experiments was probably not sufficient to cover the true optimum pH for ammonium nutrition as indicated by the work of other investigators. PIRSCHLE (**15**), TIEDJENS (**18**), and TIEDJENS and ROBBINS (**19**) have found that high pH values allow rapid absorption and assimilation of ammonium. At low pH values ammonium ions are absorbed but little assimilation occurs, according to TIEDJENS and ROBBINS.

Rhizome production and root development in these ammonium cultures were also greatest at high pH values. Plants grown in ammonium solutions at pH 6.5 had the most extensive development of lateral roots, whereas at pH 4.5 and 5.5 there was a predominance of stunted, knobby laterals, especially near the base of the main laterals. THERON (**17**) and MEVIUS (**7**) have noted this character of roots grown in acid solutions, a result here of insufficient nitrogen assimilation for proper root development. Rhizome production, which has been found by others to occur most abundantly in plants with a plentiful supply of carbohydrates, is correlated here with a pH most favorable for ammonium assimilation, as shown by top development. At high pH values, ammonium-supplied bluegrass plants exhibited a greater vegetative condition, which would seemingly indicate a low carbohydrate reserve, facts which are not in accord with increased rhizome production.

Correlations have been found by several investigators between the amount of absorption of nitrate and ammonium ions at different pH levels and changes in the pH value of the culture media. Absorption of ammonium ions from a solution brings about an increase in acidity of the solution owing to accumulation of residual anions. Thus in the case of the bluegrass plants supplied with ammonium

at pH 6.5 it is seen that month-old plants were capable of reducing the pH of the nutrient solution to 5.0 within ten hours. The rate of absorption of ammonium, as indicated by these changes in reaction of the cultures, is governed primarily by the initial pH and secondly by temperature, greatest absorption occurring at the highest temperatures. Nitrate absorption, on the contrary, is generally slower than ammonium absorption, and there being a more equal absorption of nitrate ions and their associated cations little change in pH results. In the present experiments both nitrate and ammonium solutions applied at pH 4.5 underwent an immediate change to pH 5.0. It would seem by the extreme rapidity with which both pH 4.5 solutions changed in reaction and the difficulty encountered in leaching out the cultures to the proper value, that a part of this change in acidity may be attributed to causes other than absorption of ions by the plant roots.

Nitrogen nutrition relations

Unclipped series.—Within the pH range of the experiments, the plants supplied with nitrate nitrogen showed a markedly better development than those supplied with ammonium nitrogen. The top growth of nitrate plants was superior to that of ammonium plants with respect to number and length of leaves produced and in dry weight of leaves. As has been shown under the discussion of pH relations, this superiority of nitrate nutrition over ammonium nutrition in producing an extremely vegetative plant is probably a result of more rapid assimilation of nitrate within the conditions of these experiments. Absorption rates, as inferred by changes in reaction of the culture media, are indicative of a more rapid absorption of ammonium ions than nitrate ions, but actual assimilation of nitrate ions probably occurred more rapidly under the conditions of the experiments. Tiedjens and Robbins (19) indicate that ammonium at high pH values is more available than nitrate, as evidenced by more rapid stem elongation, greater leaf area, and greater succulence in ammonium-supplied plants as contrasted with nitrate plants. The reactions of the culture solutions used in the present study were probably not alkaline enough to produce optimum growth with ammonium, and hence do not show the effects noted on other plants.

Rhizome and root production varied also with the type of nitrogen nutrition. The number of rhizomes per plant was significantly greater in nitrate plants than in ammonium plants, a fact which can be attributed partially to a more favorable carbohydrate balance under nitrate nutrition. HARRISON (5) also noted this difference in rhizome production under the two forms of nitrogen nutrition. Plants supplied with nitrate showed a greater dry weight of roots than corresponding plants supplied with ammonium, although the actual extent of root systems of the two types was similar. Measurements of root tip length showed more rapid elongation in nitrate plant roots at low and medium temperatures than in ammonium plants, but at 35° C. the latter excelled slightly.

CLIPPED SERIES.—The effects of clipping at 1 and 2 inch heights were studied on plants supplied with nitrate and ammonium nutrition at constant pH and temperature conditions. The plants used were transplants of low carbohydrate reserve and were placed under cutting treatment soon after transferral, facts which should be considered in the interpretation of the results obtained.

Weekly clipping yields showed a consistent increase over the eleven weeks experimental period under all conditions, but were more pronounced in the 2 inch clipping series than in the 1 inch plants. The greater yields of the 2 inch plants as compared with 1 inch may be attributed largely to the carbohydrate relations of the plants. With continuous and ample supply of nitrogen there is a pronounced tendency for vegetative growth, utilizing the available carbohydrates. In the two clipping series, the remaining leaves and stems allow a greater amount of carbohydrate to be built up in the 2 inch series, resulting in greater clipping yields. HARRISON (5) conducted a similar greenhouse study on plants supplied with plus and minus nitrate nutrition, and showed the advantages of carbohydrate reserves in minus-nitrate plants in maintaining a sustained yield on clipping to 0.75 inch height, as opposed to a low carbohydrate reserve in plants continuously supplied with nitrogen. His experiment covered approximately the same time interval as the one here reported, but with the slightly lower clipping height maintained by weekly clippings his plus-nitrate plants did not survive the experiment.

Greater yields were obtained from nitrate-supplied plants than from ammonium plants. On the basis of the previous studies over a

range of pH conditions, it may be concluded that nitrogen in the form of nitrate was more readily available even at pH 6.5 than was ammonium nitrogen, under the conditions of these experiments. Thus with a more readily available form of nitrogen, a greater stimulus was given to vegetative growth and yield with nitrate nutrition over the period in which the clipping studies were conducted.

Availability of nitrogen and relative rates of carbohydrate formation are also factors governing the production of new leaves. Weekly observations of increase in number of leaves on clipped plants showed a slightly greater production by nitrate-supplied plants during the first four to six weeks of the experiment, but during the latter part, ammonium plants produced a denser tuft with greater increase in number of leaves. The control plants initiated more leaves than either of the clipped series, and after the first two weeks of the experiment the ammonium-supplied controls showed the more rapid production of new leaves.

Comparison of these results with comparable portions of the previous experiments brings out several discrepancies with regard to optimum nutrition conditions for leaf production. Thus after three weeks duration, nitrate and ammonium plants had produced the same number of leaves in the clipping series controls, whereas in the three weeks experiment from May 4 to May 26, 1936, nitrate plants were decidedly superior. In the first eleven weeks series, nitrate plants showed almost twice as great an increase as ammonium plants, while the nitrate plants of the clipping series checks, grown under the same pH and temperature conditions, had but one-third as much leaf production as nitrate plants of the first series over the same time interval. However, the ammonium-supplied plants of both series showed almost identical production of new leaves during this time interval. These discrepancies may be due in part to the differences in atmospheric temperature conditions over the two experimental periods. As noted from the first experiment, the greatest number of leaves was produced under low soil temperature conditions, and thus the higher air temperatures prevailing during the clipping experiment played some part in reducing the leaf production of the nitrate check plants. The reasons are not clear for the similarity of leaf production of ammonium plants in the two experiments.

The number of rhizomes produced on the clipped plants was much smaller than on the control plants under both forms of nitrogen nutrition, a fact which has been observed by many workers and is correlated with the smaller amount of carbohydrate reserves established under clipped conditions.

Summary

1. A study was made of the effects of variation in soil temperature, pH, nitrogen nutrition, and clipping height on the development of *Poa pratensis* plants grown in sand cultures under greenhouse conditions.

2. Plants grown at temperatures of 15°, 25°, and 35° C. produced at 15° C. a tall, succulent, bushy top growth with many leaves and at 35° C. an erect, non-succulent, short top growth with few leaves. Root systems of low temperature plants were large, white, succulent, and coarsely branched, while roots of high temperature plants were small in diameter, light brown, and densely tufted.

3. Plants grown with ammonium nutrition at varied pH showed best leaf, rhizome, and root development at pH 6.5, whereas nitrate plants showed little difference within a pH range of 4.5 to 6.5.

4. Nitrate plants were superior in leaf, rhizome, and root development to ammonium-supplied plants under the conditions of temperature and pH of the experiment.

5. Plants supplied with nitrate and ammonium nutrition at constant temperature and pH and clipped weekly at 1 and 2 inch heights showed greater yields with nitrate nutrition than with ammonium nutrition. Yields over a period of eleven weeks were greatest with clipping heights of 2 inches.

The writer appreciates the valuable help and criticism of the members of the staff of the Department of Botany of the University of Chicago throughout the course of the experiments. Especial acknowledgment is due Mr. AUBREY NAYLOR for his assistance in concluding the clipping series experiment during the absence of the writer.

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LEAF GROWTH HORMONES. I. A BIO-ASSAY AND SOURCE FOR LEAF GROWTH FACTORS[1]

DAVID M. BONNER, A. J. HAAGEN-SMIT, AND F. W. WENT

(WITH SIX FIGURES)

Introduction

Few investigators have concerned themselves with a clear definition of the factors regulating leaf growth. AVERY (1), working with leaves of *Nicotiana*, was the first to correlate growth of the midrib and probably of the larger lateral veins with auxin concentration. He found the highest concentration where greatest elongation occurs. He indicated that auxin is not directly concerned in growth of the lamina, it giving no response to applied auxin. It was further pointed out by WENT and THIMANN (9) and WENT (11) that it is advantageous to distinguish between growth of the vein, which can be increased by auxin application, and growth of the mesophyll which is independent of auxin. Since growth of the latter under suitable conditions will be reflected mainly in increase in surface area, experiments on growth in leaf area may be used to analyze growth factors of the mesophyll.

Of the earlier work, that of VYVYAN (8) and of GREGORY (4) should be mentioned. VYVYAN showed that leaf growth was affected by the presence of cotyledons, and that this effect was not due to a water relationship but probably to a "food" relationship. GREGORY clearly demonstrated that leaf growth was correlated with the size of the already existing leaf area of the plant, but that this correlation could not be due to photosynthetic activity. WENT (11, 12) showed that in etiolated pea seedlings leaf growth depends upon the movement toward the growing leaves of growth factors stored in the cotyledons.

Preliminary experiments

Considering the last fact just reviewed, pea cotyledons were chosen as a source of leaf growth factors. As it was known that a

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pea does not germinate if submerged in a small quantity of water, and that if the shoot does start to elongate no leaf growth occurs when the cotyledons remain submerged, the conclusion was drawn that the water after such a leaching might contain the active principles. This leach water, or diffusate, has the additional advantages that no toxic substances are released or undue hydrolysis occurs, as happens after making the usual plant extract (**5**). It was found that such a diffusate caused appreciable growth in detached growing leaf sections, and that this growth could be simulated, in part at least, by the use of sugars and amino acids as the culture solutions.

Early experiments and observations were carried out with leaves of pea seedlings, and with the leaves of *Carica papaya*. They are discussed as the preliminary steps in the development of a satisfactory quantitative test method for leaf growth regulators. The present paper describes a satisfactory quantitative bio-assay, sources, and the general nature of the action of the special substances increasing the growth rate of immature leaves.

To determine whether the factors diffusing from the cotyledons were in any manner correlated with the effect of these same cotyledons on leaf growth in the intact plant, diffusates of Daisy and Alaska peas were compared. Equal weights of peas were diffused, and the diffusates concentrated to equal volumes. They were then tested for effect on leaf growth of Little Marvel peas, which had been found to show the greatest differences in leaf size when grafted on various varieties in transplantation experiments (**12**). Diffusate from Daisy was found far more effective than that from Alaska, comparing well with the greater leaf growth in shoots grafted on Daisy than on Alaska peas (**12**).

It was also observed that growth along the edges of cut leaf strips, from the leaves of *Carica papaya*, was much greater than in the middle when such strips were floated on the solutions. A more uniform response was obtainable by perforating the leaves with a fine steel brush. Many cells are killed and wounded by such treatment, but this allows easier penetration of the solution to the intact cells in all parts of the section, resulting in uniform growth instead of growth merely along the cut edges of the section. This effect, however, is dependent upon the kind of leaf, presumably upon the perme-

ability of the lower epidermis, and will be discussed later in this paper.

Another observation was made in connection with the effect of the solutions on the parenchymatous regions of the leaf. A differential growth rate between parenchymatous and vascular tissue should result in a characteristic bulging of the tissue. When sections containing a large unstretchable vein are floated on a pea diffusate medium they show marked bulging of the intercostal regions, since the parenchyma is growing more rapidly than the veins, yet the size of the section is determined by the length of the vein. This is just the reverse of the effect obtained by auxin treatment, when the vein bulges out between the tautly stretched mesophyll. If sections are used which have small veins, however, these will be stretched by the growing mesophyll, and the size of the section is determined by the growth of the parenchyma resulting in a plane section.

The test

In order that an object, a leaf in this case, be satisfactory for use in a quantitative bio-assay, (1) it must be reactive to the substance in question, and (2) the average of several leaves must be uniform from test to test. The following list gives the rather wide variety of leaves satisfying the first requirement, these being young rapidly growing leaves.

Brassica oleracea
Carica papaya
Lactuca sativa var. capitata
Lactuca sativa var. romana
Ludwigia
Nicotiana sylvestris
Nicotiana tabacum
Raphanus sativus (French Breakfast)
Phaseolus vulgaris (Kentucky Wonder)

That rapidly enlarging tissue was one criterion of sensitivity was further illustrated by determining the sensitivity of different portions of a young leaf of *Nicotiana sylvestris*. As shown in table 1, the proximal portion is the most sensitive, and AVERY (1) has shown it to be the most rapidly enlarging portion. Older leaves (6 cm. long) are found to be only slightly sensitive, or as a general rule totally insensitive.

In order to satisfy the second requirement, it must be possible to collect the leaves in such a way that the growth rate of the leaves used for different tests, and therefore of leaves collected at different times, is limited approximately to the same extent by the leaf growth factors of some standard crude extract containing leaf growth factors, as for example pea diffusate. It was early noticed that by collecting young leaves from certain pea varieties and from *Nicotiana* plants, some leaves would grow almost as well in sugar as in the pea diffusate, showing that their growth rate was limited primarily by sugar. On the other hand, some leaves grew little in

TABLE 1

SENSITIVITY OF DIFFERENT AREAS FROM SINGLE LEAF OF NICOTIANA SYLVESTRIS

Section cut	Growth in width above identical sections grown in 1% sucrose solution
Apex of leaf	0.8 mm.
Base of leaf	1.4
Edge of leaf	0.4
Center of leaf, including midvein	0.5
Very young leaf	1.8

sugar but a great deal in pea diffusate, showing that their growth rate was limited by some additional factor. Such leaves obviously do not meet the second requirement just mentioned. The first foliage leaves from seedling plants, however, might be much more uniform in their sensitivity since they are dependent for their growth factors upon themselves and the cotyledons, and do not receive similar factors from older leaves. This was found to be the case in *Raphanus*, and *Nicotiana* seedlings. The first foliage leaves of seedling radish and tobacco plants have been the only such leaves worked with extensively, but from the number of plants (just listed) that respond to the pea diffusate, it might be concluded that the effect of the leaf growth substances of the pea diffusate is non-specific.

It has been found that in order to obtain leaves that may be worked with satisfactorily, the plants must be grown in the light. Leaves from etiolated pea seedlings proved too small and curled to work with, and in the case of radish there is no epicotyledonary

development in plants grown in the dark. It was attempted to cause leaves to become more deficient in leaf growth hormones by allowing the plants to remain in the dark a short period before collecting the leaves, and thereby increase their sensitivity. However, no further increase in sensitivity could be obtained by allowing plants to remain in the dark for 24 to 48 hours before collection.

Raphanus plants were finally selected for the leaf test, merely because large quantities of the seed were readily obtainable, making it possible to grow considerable numbers of plants. *Nicotiana* can be used equally well, as has been shown in numerous experiments. The variety French Breakfast, obtained through the Ferry Morse Seed Company of San Francisco, California, was used. The plants are grown in the greenhouse under standard conditions. When the first foliage leaves are about 50 sq. mm. in surface area they are cut from the plant, the time after germination being about 16–18 days under our conditions. It was found necessary to adhere rigidly to these conditions, since with older leaves and with second foliage leaves the uniformity of reaction is greatly diminished. Circular disks of approximately 19.5 sq. mm. in area are cut from the leaves by punching them out with a sharp cork borer. The variation in size from disk to disk is negligible. The disks are carefully washed in distilled water, and shaken for five minutes on an electric shaker to ensure thorough mixing of all of the sections. Fifteen to twenty sections are then placed in Syracuse dishes containing 2 cc. per dish of medium. This number of sections was found to be sufficient to reduce the variation in sensitivity from dish to dish so that a reproducibility of 2–3 per cent can generally be obtained. The dishes are placed in an incubator at $25° \pm 0.5°$ C. for 30 hours. This length of time was found to give a maximum difference in area of about 30 per cent between control sections and sections grown in standard pea diffusate medium. Allowing the test to run for longer than 30 hours was found undesirable, since the difference in area was enhanced little and the medium becomes badly contaminated.

Several types of direct measurements were attempted for measurement of leaf growth. Leaf strips were used, their width being measured under a binocular with an eyepiece micrometer. These meas-

urements were abandoned, however, since owing to the resistance to stretching of the larger veins the growth of the sections was rather irregular. This made the personal equation too great for satisfactory quantitative work. In order to gain sufficient reproducibility it was found necessary to use a considerable number of sections, making direct measurements of area increase very laborious.

Two equally reliable methods for measuring the increased growth were therefore worked out and used: (1) direct determination of the total wet weight of the sections from one dish; and (2) measurement of the total surface area of the sections from one dish. The method of direct determination of wet weight is the faster, and therefore

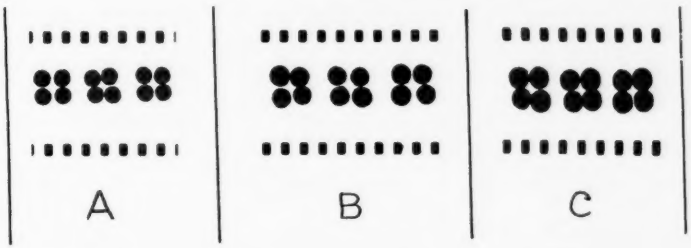

FIG. 1.—Film arrangement for determination of leaf section surface area: *a*, sections of radish leaves grown 24 hours in water; *b*, grown in 1% sucrose; *c*, grown in pea diffusate medium plus 1% sucrose.

used in routine testing, while the alternative method may be used as a check. The method of obtaining the wet weight is as follows: All the sections from a single dish are placed upon a piece of blotting paper, taking care that the sections are separated from one another. They are dried on this paper for about 1 minute, and a definite number then transferred to a piece of dry filter paper of known weight, their weight then being determined to a tenth of a milligram. These procedures are done in a uniform manner, so that the error from varying degrees of dryness appears to be negligible. For measuring the total surface area a photoelectric cell is used. The cell is arranged in such a manner that a film may be passed between it and a suitable light source directly above the cell. By such an arrangement, only light transmitted by clear areas in the film is caught by the cell. The galvanometer deflection is then a measure of the

amount of clear area on the film. Application of this principle for measurement of leaf section areas is as follows: The sections are first

TABLE 2

COMPARISON OF ACTIVITIES MEASURED WITH PHOTOELECTRIC CELL, AND OF DIRECT MEASUREMENT OF FILM UNDER MICROSCOPE WITH EYE PIECE MICROMETER

Sections from Nicotiana leaves in	Method	
	Photoelectric cell	Direct
Water	100	100
Sucrose (1%)	111	110
Sucrose (1%)+pea diffusate mg. dry wt./cc.		
1	153	150
0.1	143	142

TABLE 3

COMPARISON OF ACTIVITIES DETERMINED BY WET WEIGHT DETERMINATION, AND MEASURING SURFACE AREA WITH PHOTOELECTRIC CELL

Sections from Raphanus leaves in	Method			
	Wet weight determination		Area determination	
	mg./16 sections	Relative to water controls	Galvanometer deflection in arbitrary units	Relative to water controls
Water	42.8	100	20.3	100
Sucrose (1%)	50.3	117	23.4	115
Sucrose (1%)+pea diffusate mg. dry wt./cc.				
1	63.5	149	30.8	151
0.5	62.2	145	30.4	149
0.2	61.5	144	29.6	146
0.02	59.2	139	29.0	143

dried as before and arranged on a piece of thin glassplate in sets of four, with lines of demarcation between the sections from different

dishes. A shadow photograph of the sections is then made on a strip of 32 mm. positive film, using a very weak exposure and an alkaline hydroquinone developer to insure maximum contrast (fig. 1). An entire experiment of 20–30 dishes may be photographed on two strips of film, so that error from varying degrees in the density of the film is minimized. In addition two or three standard areas (disks cut from a piece of steel rod) are photographed on each strip of film, so that they may be used to calibrate the measurements to the same zero point on the galvanometer for each individual film. The film is then passed over the photoelectric cell with a direct light source

TABLE 4

EFFECT OF pH ON LEAF TEST

SECTIONS IN	pH OF MEDIUM	GROWTH RELATIVE TO SUGAR CONTROLS
Sucrose (1%)	Unbuffered	100
Sucrose	4.0	100
Sucrose (1%)+pea diffusate		
mg. dry wt./cc.	Unbuffered	110
10	7.0	110
10	6.0	111
10	5.0	110
10	4.0	109

above it. The cell catches only the light transmitted through the clear spaces on the film, these clear areas corresponding to the surface area of the original leaf sections. The galvanometer deflection is noted, and the difference between the readings is a measure of the difference in surface area of the original leaf sections. By this method the measure of activity is indicated by the increase in total surface area of twenty leaf sections grown in the crude extract over that of suitable controls. The correspondence between areas is measured in this manner, and the determination of areas by measuring the photograph under a microscope with an eyepiece micrometer is very good (table 2). The agreement between the method of determination of the wet weight of the sections and of determination of their surface area is shown in table 3.

The test is independent of pH from a pH of 4.0 to a pH of 7.0

(table 4). Below a pH of 4.0 the medium becomes toxic, owing to the external pH changing the internal sufficiently to cause damage to the tissue. The test is light independent, but is dependent on temperature, the sensitivity being less at lower temperatures.

Sources

It has been possible to obtain extracts, active in the test just outlined, from several different sources. These fall into two general categories: (1) seed diffusates, and (2) leaf extracts.

The method of obtaining a seed diffusate is as follows:

a) Sterilization of the seed.—

1. Wash the dried seeds 10–12 times in tap water.
2. Wash 6–8 times in distilled water.
3. Place in a container from which the diffusate is to be collected (a large separatory funnel has been found excellent for this purpose).
4. Soak 2 minutes in 95 per cent alcohol.
5. Rinse 5–6 times with sterile (boiled) distilled water.
6. Soak 30 minutes in 0.1 per cent mercuric chloride.
7. Rinse six times with sterile distilled water.

b) Obtaining the diffusate:

After this treatment the seeds are covered with sterile water and left for 12 hours, then drained off and placed in fresh sterile water. The water that has been drained off is concentrated in vacuo at 30° C.

A general analysis of such a diffusate obtained from pea seeds is shown in table 5.

Pea seeds treated in this manner remain sterile indefinitely, and in the case of the leaf growth hormones, as for biotin (**5**), they continue to be given off at a constant rate for a very long time. Even after a month of diffusing, leaf growth hormones are given off at nearly the same concentration as when the diffusion was first started. Diffusates active in the leaf test have been obtained in a fashion similar to that just described from corn, radish, and pea seeds. It should be noted that the diffusates from these three widely different plants are all active in causing an increased growth rate in

a radish leaf. This lends additional support to the view that the active principle, or principles, in each of the three diffusates is probably the same, and that a common hormone governs the leaf growth in a large variety of plants.

TABLE 5*

GENERAL ANALYSIS OF PEA DIFFUSATE

Fraction determined	Percentage dry weight
Total nitrogen (micro-dumas)	4.36
Total nitrogen(micro-Kjeldahl)	3.2
Protein nitrogen	0.0
Non-protein nitrogen	3.2
Ammonia nitrogen	0.3
Amino nitrogen	1.9
Amide nitrogen	0.05
Polypeptide nitrogen	0.02
Reducing sugars	5.6
Sucrose	40.5
Non-carbohydrate reducing substances	10.5
Ascorbic acid	0.0

* We are indebted to Prof. H. Borsook and Mr. W. McRary for the nitrogen fraction determinations.

No active extract has been obtained by a water extraction of fresh ground-up leaves. However, by placing the leaves intact in sterile water and allowing them to remain in it, at a low temperature to keep down infection, it is possible to obtain a leaf diffusate that is active. By allowing the leaves first to soak in ether for a few days, thereby inactivating any destructive enzymes, a water extract of these leaves is active in the leaf test. The extract obtained by this second method is much more active than the leaf diffusate from mature *Nicotiana* leaves. From young palm leaves (*Washingtonia filifera*) on the other hand, an exceedingly active extract may be prepared by merely allowing the leaves to soak in sterile water for a week, draining the water off, and concentrating this diffusate in vacuo. Active leaf extracts and diffusates have been prepared from leaves of *Nicotiana*, *Raphanus*, and *Washingtonia*. Yeast extract was found to be very active in the leaf test. Its activity could not be explained as due to thiamin, riboflavin, ascorbic acid, or biotin, these substances being inactive when tested by themselves.

Units and standards

The response of leaf sections to various substances is being studied in this laboratory and further reports will be made regarding the nature and chemistry of the leaf growth hormones, but it is desirable to discuss here certain limiting factors. Sugars, as mentioned earlier in this paper, proved to be active in the leaf test, and figure 2 shows the activity concentration curve obtained for sucrose. The particu-

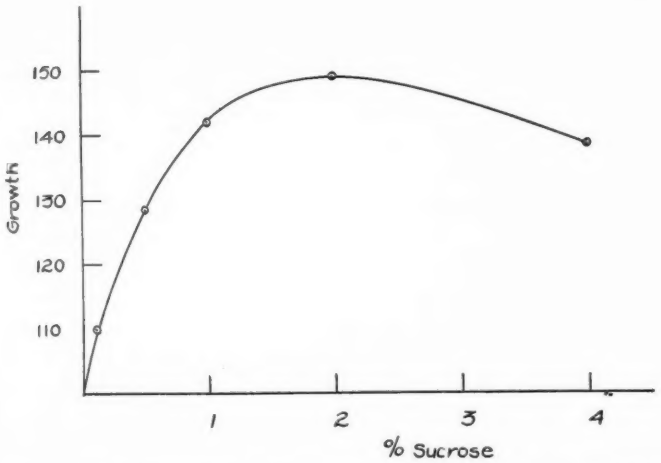

FIG. 2.—Effect of sucrose on leaf growth expressed as growth above growth of sections in water.

lar type of sugar used plays an important role. Both sucrose and glucose have the effect shown in figure 2. Mannose and galactose on the other hand give no appreciable effect. This might indicate that the effect of sugars is not merely an osmotic one. The diffusate by itself has a high activity, as shown in figure 3. To remove the possibility of a sugar being the limiting factor in the growth reaction, a basic sugar medium was added to all extracts tested and the growth compared with that of sections grown on sugar alone. The sugar selected was sucrose, it giving a good reaction (fig. 2); and as seen from table 5, pea diffusate contains 40 per cent sucrose calculated as percentage of its dry weight. Two per cent by weight of sucrose

gives optimum growth when sucrose is used by itself. By trying several dilutions it was found that in the presence of pea diffusate

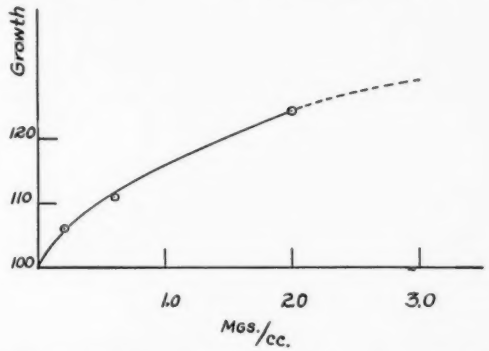

FIG. 3.—Effect of pea diffusate on leaf growth. Concentration of pea diffusate in terms of mg. dry weight of pea diffusate per cc. of water. Growth expressed as growth above growth of sections in water.

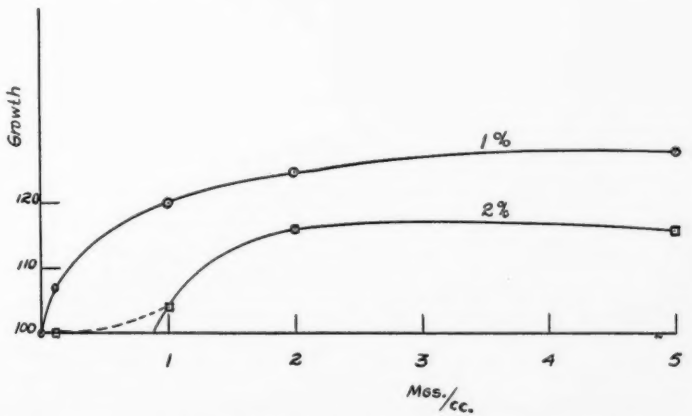

FIG. 4.—Growth of leaves in pea diffusate with different concentrations of sucrose. Growth expressed as growth above growth of sections grown in appropriate sucrose concentration.

optimum growth was obtained using a 1 per cent by weight sucrose solution (fig. 4). All extracts are now tested in a medium consisting of 1 per cent sucrose, and activity is measured in terms of the added

growth beyond that of sections grown in this basic medium. In order that activities be compared from day to day it is necessary to have a solution of "standard activity." It was found convenient to define such a solution as one containing 10 mg. dry weight of pea diffusate per cubic centimeter of 1 per cent sucrose solution. This solution is tested in a standard dilution series, consisting of four dilutions from 50 to 2 per cent (fig. 5). It has been found that if the diffusate is concentrated to a syrup and stored in the cold, standard solution preparations from this concentrated diffusate give a relatively constant activity over a period of 2–3 months. The standard solution itself, however, must be made up fresh every day or two from concentrated diffusate, since after that time its activity becomes variable. That such a solution may be used as a standard is due to the fact that standard solution preparations made from concentrated diffusates which were prepared at widely different times but from the same variety of pea have nearly the same activity.

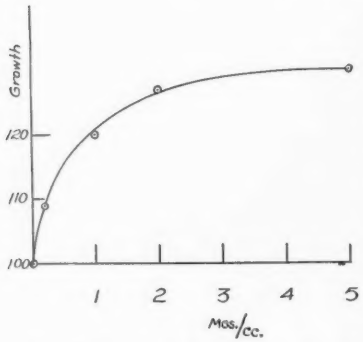

FIG. 5.—Standard dilution series. Growth expressed as growth above growth of sections grown in 1% sucrose solution.

In calculating activities of extracts it is necessary to select an arbitrary unit. We have defined one leaf unit (l.u.) as the activity of a solution containing 0.1 cc. of the standard solution; that is, 1 mg. dry weight of pea diffusate per cc. of 1 per cent sucrose solution. The number of leaf units an extract contains is determined by plotting the percentage increase of growth (growth above that of the sugar controls) against concentration in mg./cc. Graphically the concentration of the extract which shows the same activity as the standard solution at a concentration of 1 mg. dry weight of pea diffusate per cc. of 1 per cent sucrose solution is determined. This concentration gives the milligrams dry weight of the extract per leaf unit.

The preceding paragraphs have shown the effect of leaf growth

factors for a relatively short time on a leaf section. It would be desirable to determine the effect of these same factors on an intact leaf over a much longer time. For this purpose pea seedlings were grown sterilely in test tubes containing nutrient medium. Twelve days after germination the foliage leaves were cut off sterilely, and transferred to flasks containing suitable medium. Three series of medium were used: (1) sterile water; (2) a balanced inorganic

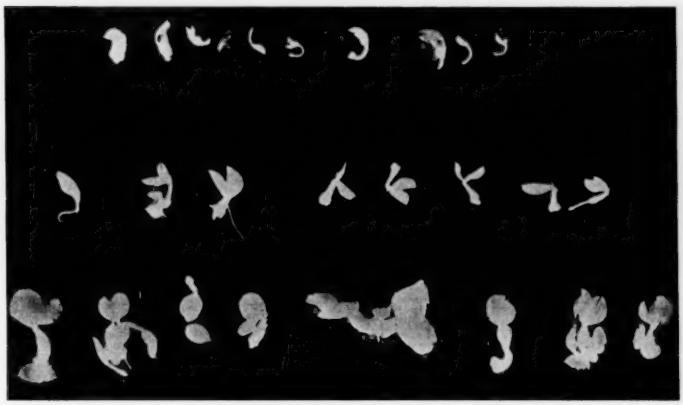

FIG. 6.—Growth of leaves excised from etiolated pea seedlings, cultured 1 month. Top row: in water; middle row: in inorganic salt medium plus 1% sucrose; bottom row: in inorganic salt and 1% sucrose medium, plus 1% standard pea diffusate solution.

medium (3) plus 1 per cent sucrose; and (3) the same as (2) plus 0.2 mg. pea diffusate per cc. of medium. They were allowed to grow in these media for 1 month in darkness, after which they were taken out and a shadow photograph made of them. Figure 6 indicates that over a longer period of time the effect of pea diffusate in increasing the growth of leaves is even more pronounced than in the leaf test just described. The leaves have grown more than they would have done under comparable conditions (darkness) on the plant.

Nature of growth response

Histological studies have been started on both *Nicotiana* and *Raphanus*. Disks were grown in a manner similar to those grown

in a regular leaf test, but were notched with regard to a known orientation of the midvein. The sections were killed with Navashin's solution under a slight vacuum to allow quick penetration of the fixative. They were then dehydrated in alcohol and tertiary butyl alcohol, and finally imbedded in a beeswax paraffin mixture. The sections were cut transversely and longitudinally at a thickness of 15 μ, and were stained with a modified Flemming's triple stain.

Preliminary studies of both *Nicotiana* and *Raphanus* indicate that a gross enlargement in area and in thickness of the sections has taken place. Before killing the sections, measurements of area were made under a microscope with an eyepiece micrometer. They showed a 20 per cent increase in area of sections grown in pea diffusate over sections grown in sucrose. Measurements of thickness were made after mounting the sections. The thickness was uniformly greater (measured from epidermis to epidermis) in leaves grown in pea diffusate than in sucrose. It is of interest also that in radish and tobacco the increase in thickness was rather uniform throughout the length of the sections. This would indicate that the growth factors penetrated uniformly through the epidermis, and that in these two cases lack of penetration was not a limiting factor, as mentioned earlier for *Carica papaya*.

The histological studies also indicate a general trend toward a greater degree of cell enlargement in sections of both radish and tobacco grown in the pea diffusate than in sucrose. There was evidence not only of cell enlargement in sections grown in the crude hormone, but also very marked increase of intercellular spaces of the spongy parenchyma.

Discussion

It has previously been demonstrated that elongation of stems is controlled by a hormone auxin (**10**) of known chemical constitution (**6**), and the growth of roots is controlled by a hormone thiamin (**2, 5**) of known chemical constitution (**13**). The present investigation indicates that the growth of leaves is also controlled by a hormone. This hormone is *non species* specific in nature, and the hormone is probably common to a considerable variety of leaves. The necessary preliminary steps in the determination of the chemical

nature of this substance (the finding of a suitable crude source of the hormone and a suitable bio-assay with which to follow its isolation) are reported in this paper. In this connection it is interesting that recently STAUFFERT (7) also has concluded that vein growth and mesophyll growth are phenomena which are differentially affected by genes. For the laciniate form of *Chelidonium majus* it was concluded that this character apparently does not reflect a morphogenetic gene but results merely in a decreased mesophyll growth.

Summary

1. The preliminary experiments in the formulation of a bio-assay for leaf growth factors and in the finding of a crude source of these factors are described.

2. A quantitative bio-assay for hormones controlling the growth of leaves is described. The general type of leaves and the method of using them are described, together with suitable methods for measuring the increased growth of isolated leaf sections.

3. The sources of these growth factors may be classified in two general types, seed diffusates and leaf extracts. Yeast extract was also found to be a good source of these factors.

4. The basic medium of the leaf test is described as a 1 per cent sucrose solution. This is used in order that the growth rate of the leaves may not be limited by sugar. Units and standards in the leaf test are described. A solution of "standard activity" is defined as well as a "leaf unit."

5. The general nature of the growth response to pea diffusate is discussed. In both *Raphanus* and *Nicotiana* there is an increase in area of the sections grown in pea diffusate, and an increase in thickness. Cell enlargement also appears to be greater, with a separation of the spongy parenchyma and formation of more intercellular air spaces.

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EFFECT OF VARIATION IN TEMPERATURE DURING PHOTOPERIODIC INDUCTION UPON INITIATION OF FLOWER PRIMORDIA IN BILOXI SOYBEAN

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Introduction

Photoperiodic induction in Biloxi and other varieties of soybean is influenced greatly by factors other than photoperiod. Age of plant at the time of treatment (2), intensity of light during treatment (1), and variety of soybean used, have been shown to contribute to variations in the results obtained.

Many studies dealing with the influence of temperature upon flowering in various kinds of plants have shown that this factor, operating alone or in conjunction with photoperiod, may exert a tremendous influence upon the time and extent of flowering. But few, if any, of these studies have been directed specifically at the interrelation of temperature and photoperiod during photoperiodic induction.

In the present work on this inter-relation a careful study has been made of the extent of floral initiation following various treatments. Along with this work, studies have been made of the differences in amount of growth and change in chemical composition brought about during the period of induction.

Literature review

GARNER and ALLARD, studying the photoperiodic response of soybeans in relation to temperature and other environmental factors, state: "The available evidence seems to indicate that under field conditions at Washington variations from year to year in date of flowering of both early and late varieties of soybeans when planted on any particular date are due chiefly to differences in temperature, while length of day is the primary external factor responsible for the fact that one variety is always relatively early and another late in

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attaining the reproductive stage" (4). CHROBOCZEK (3), working with beets, has shown that favorable conditions of light and temperature are essential to the development of a fertile inflorescence as well as to the initiation of flower primordia. By regulating the temperature and the photoperiod, the time normally required for seed-stalk formation could be greatly shortened or materially lengthened.

STEINBERG and GARNER (13) report that the critical photoperiod for flowering in soybean, *Rudbeckia bicolor*, and beet may be altered to a limited degree by temperature, and conversely, the favorable temperature range for flowering may be shifted by the action of the photoperiod.

KNOTT (7), studying the effect of temperature on the photoperiodic response of spinach, found that with photoperiod fixed at 15 hours per day seed-stalk elongation occurred sooner if temperature during treatment was held at 60° to 70° F. than it did if held at a higher or lower range. ROBERTS and STRUCKMEYER (11) found that the response of many varieties of plants to photoperiod could be materially altered by varying temperature. Although they included four varieties of soybean in their studies, the only record of their results consists of a text figure dealing with the variety Illini. This shows that flowering and fruiting on short day can be delayed by growing the plants at low temperature.

GILBERT (5) studied the interrelation of length of day and temperature in *Xanthium pennsylvanicum* and reported that temperature influenced the time of production of flower primordia. He employed both long and short photoperiod but the long photoperiod averaged only 14.6 hours. Initiation of floral primordia under GILBERT'S long day condition would therefore be expected, since HAMNER and BONNER (6) report that the critical photoperiod for this species is approximately 15.5 hours. They likewise showed that temperature influences floral initiation in *Xanthium*. Both high and low temperatures applied during the 16-hour dark period inhibited initiation but had no marked influence when applied during the 8-hour photoperiod.

While this literature review presents only part of the work that has been done on the interrelationship of temperature and photoperiod, it shows that many of these studies were primarily concerned

with flowering, fruiting, or seed-stalk production, and were not restricted specifically to the role that temperature plays in the initiation of flower primordia.

Material and methods

Biloxi soybeans were grown in the greenhouse in 4-inch pots, with the natural photoperiod extended to midnight with Mazda light of about 50 foot candles. When they were four to six weeks old they were transferred to a series of control rooms where they received photoperiodic induction at various controlled temperatures. At the time of transfer, numerous plants were dissected and, as has been found in many previous experiments, no flower primordia were present. Because the number of plants examined was large it seems safe to assume that the experimental plants were likewise vegetative at the beginning of treatment.

The induction treatments were given in four control rooms operated at 55°, 65°, 75°, and 85° F. respectively. These temperatures were controlled to within ±2° F. Each room was illuminated with an arc light burning Sunshine carbons and furnishing more than 1000 foot candles of light at the plant surfaces. Accompanying this arc light, Mazda light of approximately 160 foot candles intensity was added simultaneously. When such an amount of Mazda light was employed in previous experiments where soybeans were grown from seed under artificial light, it was found that plants of considerably better size and quality were obtained than when the arc alone was used.

The photoperiods used were 8 hours in some experiments and 16 in others. Various temperature combinations were obtained by moving the plants from one room to another at the beginning and end of the photoperiod. Since each room was maintained at a constant temperature it was possible to obtain sixteen combinations of night and day temperature. In four of these combinations the night and day temperatures were identical; in the other twelve the night temperature differed from the day. The duration of the differential temperature treatments was three and five days in certain of the experiments and five and ten days in others. When studies of flower bud initiation were to be made, the plants were returned to the green-

house and allowed to continue their development on 16-hour photoperiods. Such a treatment when applied to the Biloxi soybean may result in the formation of flower primordia, after which the plants resume the differentiation of vegetative structures; therefore the number of flower primordia formed gives a measure of the effectiveness of the treatment applied. In experiments involving 8-hour photoperiods the plants were dissected 16 days after induction treatments were completed. The plants for chemical analyses and some of those for morphological examination were collected immediately at the end of the induction treatments.

Experimental results with 8-hour photoperiod

Morphological and growth responses

Plants were subjected to 8-hour photoperiods at each of sixteen combinations of temperature during the light and dark periods. For this study 480 plants were selected for uniformity from approximately 800. These were divided into thirty-two lots of fifteen plants each. Sixteen of these lots were subjected to photoperiodic induction for three days and the remainder for five. This experiment was repeated later with another set of plants that were of approximately the same stage of development at the beginning as those used in the first experiment.

Three weeks after the beginning of induction the plants were dissected. Dissection of the 480 plants of each group required about three days, but all of the plants were harvested at the same time, defoliated, and stored at low temperature with their lower ends in water until examined. As they were dissected, records were made for each plant of the total number of nodes in the main axis and the positions on the main axis of all buds containing flower primordia.

In most of the experimental lots the induction treatment was sufficient to cause differentiation of a few flower buds by most of the plants. The number of plants in each treatment that initiated flower primordia served as one index of the effectiveness of the treatment. The treatments were purposely made brief so that after return of the plants to 16-hour photoperiod the differentiation of vegetative structures would again be resumed. Since the plants were not dissected until sixteen days after completion of the induction treatments, there

was sufficient time for differentiation of many new buds, in the axils of younger leaves. In all treatments the youngest of these newly formed buds were made up of vegetative structures only. The buds containing flower primordia were located below these, and their number therefore served as an additional measure of the effectiveness of the induction treatment applied. The dissection results were analyzed by the method of analysis of variance as described by SNEDECOR (12).

The total number of plants bearing flower primordia in the first run was not significantly different from the total number in the second. There were 259 plants with flower primordia in the first and 236 in the second. There was a marked difference, however, between the lots receiving 3-day and 5-day induction periods. In the 3-day group 33 per cent of the plants initiated flower primordia, while in the 5-day group approximately 70 per cent showed floral initiation.

The most striking effect of temperature variation during induction was caused by low temperature during the dark period (table 1). When the temperature during the photoperiod was constant, fewer plants initiated flower primordia at 55° F. than at any other temperature during the dark period. The same relationship was found with respect to the total number of buds per lot containing flower primordia, mean number of buds per plant containing flower primordia, and mean number of nodes in the main axis of each plant. Temperatures during the dark period of 65° and 75° F. were most favorable so far as initiation of flower primordia was concerned. Under the conditions of the experiment, 85° F. was above the optimum for floral initiation and also for the most rapid differentiation of new nodes in the main axis.

Variations in temperature during the photoperiod also produced differences in floral initiation. With increase from 55° to 75° F. there was a trend toward increase in number of plants bearing flower primordia and number of flower primordia per plant. This was also true with respect to the total number of nodes formed.

In general the differences resulting from variation in temperature during the dark period were greater than those associated with variation in temperature during the photoperiod (table 1). These greater

TABLE 1

EFFECT OF COMBINATIONS OF TEMPERATURE DURING 5 DAYS' INDUCTION WITH 8-HOUR PHOTOPERIODS UPON VARIOUS MORPHOLOGICAL CHARACTERISTICS OF BILOXI SOYBEANS*

Temperature during dark period (°F.)	Temperature during photoperiod (°F.)			
	55°	65°	75°	85°
	1. Total plants per lot† bearing flower primordia			
55°	0	4	10	8
65	26	26	30	30
75	25	30	29	29
85	16	21	25	25
	2. Total buds per lot† containing flower primordia			
55	0	5	17	9
65	55	66	115	112
75	65	97	123	110
85	29	50	74	89
	3. Mean number of buds per plant‡ containing flower primordia			
55	0	1.3	1.7	1.1
65	2.1	2.5	3.8	3.7
75	2.6	3.2	4.2	3.8
85	1.8	2.4	3.0	3.6
	4. Mean number of nodes per plant in main axis			
55	18.0	17.8	18.5	18.8
65	18.3	18.6	19.0	19.1
75	19.0	19.4	19.6	19.5
85	18.5	18.9	18.9	19.0

* Data based on dissections made 3 weeks after beginning of induction.

† Each lot contained 30 plants.

‡ Total buds per lot containing flower primordia divided by total plants per lot bearing flower primordia.

Differences required for odds of 19 to 1: (1) 6.2; (2) 18.0; (3) 0.8; (4) 0.5.

differences are probably to be attributed in part to the fact that the dark periods were twice as long as the photoperiods.

The differences in nodes per plant among the various treatments shown in table 1 were not great, but analysis of variance indicates that they were highly significant. The differences associated with variation in temperature during the photoperiod exceeded the 1 per cent level of significance, and those associated with differences in temperature during the dark period were found to be considerably greater. In general the treatments unfavorable to floral initiation were also unfavorable to differentiation of new nodes in the main axes of the plants. This apparent correlation suggests that the reduction in number of plants initiating flower primordia in certain treatments was brought about largely by the fact that initiation of new structures of any kind in these lots was proceeding at a very slow rate. The data of table 1, however, give the relative number of nodes present more than two weeks after the induction treatments were finished, and therefore include not only differences that arose during the induction but also any that arose subsequent to it.

Since additional data were needed to determine the extent of differences in total nodes existing immediately after induction, another experiment was performed in which dissections were made at the end of the 5-day treatment.

Special care was taken in setting up the experiment to make it as sensitive as possible, because the total number of nodes differentiated in five days would be small, making the differences between treatments correspondingly smaller. The number of temperature treatments was reduced to four to permit use of a greater number of plants per lot. Plants far in excess of the number needed in the experiment were grown on a greenhouse bench for about five weeks prior to the beginning of induction. From this supply lots of five plants each were selected, special care being taken that the individuals of each group were as nearly identical in size and stage of development as possible. The plants were labeled and one was then placed in each of the five experimental groups. This process was repeated sixty times, thereby providing five uniform lots of sixty plants each.

Four of the lots were subjected to five days' induction with 8-hour photoperiods at constant temperatures of 55°, 65°, 75°, and 85° F.

respectively. The fifth lot was dissected at the time the induction treatments of the other four were started. At the end of five days the four lots were harvested and dissected. Differences in total nodes between the initial lot and each of the other four gave a measure of the amount of differentiation that occurred in each group. The results were subjected to variance analysis and are presented in table 2.

TABLE 2

EFFECT OF VARIOUS TEMPERATURES UPON NUMBER OF NODES PER PLANT DIFFERENTIATED IN MAIN AXIS DURING 5 DAYS' INDUCTION WITH 8-HOUR PHOTOPERIODS

Lot no.	Temperature during induction (°F.)		Mean no. of nodes per plant at time of dissection*	Mean gain in nodes per plant during 5 days' induction
	Photoperiod	Dark period		
1	55°	55°	16.13	0.26
2	65	65	16.95	1.08
3	75	75	17.66	1.79
4	85	85	17.57	1.70
5	Control; no induction		15.87	.....

* Lots 1, 2, 3, and 4 dissected at close of 5 days' induction; lot 5 at beginning of induction. Difference of 1.04 nodes per plant required for odds of 19–1.

The differences in total nodes per plant that arose between different treatments were not greatly different from those found in corresponding lots of the previous experiment. In both experiments the lowest value was obtained at 55° F. and there was an increase in number with higher temperatures. Differentiation of new structures at 55° F. was practically at a standstill during the induction period. At the most effective temperature nearly two nodes were added during the five days. The data seem to indicate therefore that the differences between lots in total number of nodes per plant (table 1) arose during the time of induction rather than subsequent to it.

When the plants used in the first dissection studies were harvested, various internodes of every plant were measured and fresh and dry weight determinations of leaves and stems of each lot were made. Analysis of length measurements indicated that the response made to treatments varied greatly from one part of the stem to another.

At the time of harvest seven separate measurements of stem length were made of each plant. The first six of these were of the first six internodes individually, the seventh included the remainder of the stem above the sixth internode. No significant differences were present in the first three internodes, which suggests that elongation in that portion of the stem was complete before the start of the experiment.

It was found that differences in temperature during the photoperiod produced significant differences in the lengths of the fourth and fifth internodes but not in the lengths of the sixth internodes and terminals. In the two former structures there was a trend toward increased length with each increase in temperature. On the other hand, temperatures during the dark period induced differences in length in each of the four uppermost regions measured. In the fourth internode greater length was associated with higher temperature. In the fifth the differences were small but in the reverse direction; longest internodes were produced at lowest temperatures. This same relationship was also found in the sixth internode but the differences were even greater. In the remainder of the stem above the sixth internode there was an increase in length for each increase in temperature during the dark period up to 75° F. At 85° F., however, the terminals were shorter than at 75° F.

These data were collected three weeks after the beginning of the induction treatments and they therefore reflect not only differences originating during induction but also such differences as developed subsequent to and as indirect effects of the induction treatments.

Data from another experiment, in which the actual gains in length made by certain internodes during the 5-day induction period were measured, indicate that these indirect effects may account for some of the differences observed. In this experiment the internodes of five plants from each of sixteen treatments were measured at the beginning of the experiment and again five days later at its termination. In these plants no significant increase in length of the first four internodes occurred during this time. The fifth internode nearly doubled in length during the five days of induction, however, and the remainder of the stem above the fifth internode more than quadrupled its length.

Although the fifth internode made considerable growth, the increases in length were fairly uniform for all lots except two, which were considerably lower than the rest. Both of these received a 55° dark period, but one was at 55° and the other at 85° F. during the photoperiod. Considerable differences occurred in length of terminals of the various lots. In almost all cases an increase in temperature during either the light or dark period resulted in increased length of this part of the stem.

In addition to the length measurements and dissection studies reported on these plants, measurements were made of changes in leaf area during induction treatment. These data were obtained by blueprinting certain young expanding leaflets at the beginning and end of a 5-day induction period. The area of these prints was later determined photoelectrically by a leaf-area measuring apparatus similar to the one described by MITCHELL (9).

At each of the four temperatures used during the photoperiod the greatest increase in area occurred when the temperature during the dark period was 75° and least when it was 55° F. At 85° somewhat smaller increases in area occurred than at 75° F. With temperature during the dark period held constant, differences in area resulting from variations in temperature during the photoperiod were not great except at 55°, when the results were consistently lower.

A study was made of the differences in fresh and dry weight at the end of a 5-day induction period of a series of plants that received the same temperature treatments as those reported in table 1. These plants were about five weeks old at the beginning of treatment. In selecting them for the experiment special care was taken to insure uniformity among the different lots. The plants were harvested at the end of the dark period following the fifth 8-hour photoperiod, and the leaves were removed from the stems. The results are shown in table 3.

The percentage of dry weight in the leaves and stems was influenced greatly by the temperature during the dark period but not by that of the photoperiod. When the temperature during the photoperiod was maintained constant, there was a progressive decrease in the percentage of dry weight with each 10° rise in temperature during the dark period. The only exception was a reversal of two of

the lots subjected to photoperiods at 85° F. The amount of floral initiation as influenced by temperature during the dark period, on

TABLE 3

EFFECT OF COMBINATIONS OF TEMPERATURE DURING 5 DAYS' INDUCTION WITH 8-HOUR PHOTOPERIODS UPON FRESH AND DRY WEIGHT PER PLANT AND PERCENTAGE OF DRY WEIGHT OF BILOXI SOYBEANS

Temperature during dark period (°F.)	Temperature during photoperiod (°F.)											
	Leaves and stems				Leaves				Stems			
	55°	65°	75°	85°	55°	65°	75°	85°	55°	65°	75°	85°
	1. Fresh weight (gm. per plant)											
55°	11.67	12.23	12.90	12.23	7.50	7.87	8.10	7.67	4.17	4.37	4.80	4.57
65	12.23	13.67	14.00	14.77	7.87	8.67	8.83	9.17	4.37	5.00	5.17	5.60
75	12.47	14.67	14.57	14.37	7.87	9.20	9.20	8.73	4.60	5.47	5.37	5.63
85	12.50	13.27	13.30	14.03	7.63	8.30	8.30	8.57	4.87	4.97	5.00	5.47
	2. Dry weight (gm. per plant)											
55	1.52	1.64	1.68	1.65	1.03	1.13	1.13	1.10	0.49	0.50	0.55	0.55
65	1.38	1.56	1.64	1.71	0.93	1.05	1.10	1.13	0.45	0.51	0.54	0.58
75	1.37	1.60	1.61	1.55	0.92	1.07	1.07	1.00	0.46	0.54	0.54	0.55
85	1.36	1.41	1.46	1.53	0.90	0.93	0.97	1.00	0.46	0.47	0.50	0.53
	3. Percentage dry weight											
55	13.06	13.38	13.02	13.46	13.78	14.41	13.99	14.35	11.76	11.53	11.39	11.97
65	11.31	11.41	12.74	11.60	11.86	12.12	12.45	12.36	10.31	10.20	10.52	10.36
75	11.02	10.93	11.03	10.81	11.60	11.59	11.59	11.45	9.93	9.82	10.06	9.82
85	10.85	10.60	11.00	10.93	11.79	11.24	11.65	11.67	9.38	9.53	9.93	9.76

Differences required for significance at odds of 19 to 1:

1. Fresh weight:		2. Dry weight:		3. Percentage dry weight:	
Leaves and stems	2.2	Leaves and stems	0.17	Leaves and stems	0.38
Leaves	0.84	Leaves	0.14	Leaves	0.47
Stems	0.46	Stems	0.05	Stems	0.37

the other hand, showed a maximum at 75°. It would seem therefore that the two responses are not directly correlated.

No data were obtained as to the actual rates of photosynthesis or

respiration. It was known, however, that the dark period in which respiration predominated was twice as long as the photoperiod dur-

TABLE 4

EFFECT OF COMBINATIONS OF TEMPERATURE DURING 5 DAYS' TREATMENT WITH 16-HOUR PHOTOPERIODS UPON FRESH AND DRY WEIGHT AND PERCENTAGE OF DRY WEIGHT

Temperature during dark period (°F.)	Temperatures during photoperiod (°F.)											
	Leaves and stems				Leaves				Stems			
	55°	65°	75°	85°	55°	65°	75°	85°	55°	65°	75°	85°
	1. Fresh weight (gm. per plant)											
55°	12.77	15.27	15.17	14.97	8.27	9.90	9.70	9.47	4.50	5.37	5.47	5.50
65	14.27	15.47	17.47	16.30	9.27	9.97	11.17	10.20	5.00	5.50	6.30	6.10
75	14.83	15.40	17.00	16.83	9.73	9.77	10.90	10.40	5.10	5.63	6.10	6.43
85	14.03	15.73	16.70	16.07	9.07	10.03	10.60	10.13	4.97	5.70	6.10	5.93
	2. Dry weight (gm. per plant)											
55	2.05	2.51	2.29	2.35	1.37	1.70	1.50	1.53	0.68	0.81	0.79	0.82
65	2.13	2.30	2.41	2.25	1.42	1.53	1.57	1.43	0.72	0.77	0.85	0.82
75	2.14	2.14	2.32	2.12	1.40	1.38	1.50	1.32	0.74	0.75	0.82	0.81
85	1.91	2.11	2.20	2.11	1.23	1.37	1.40	1.33	0.68	0.74	0.80	0.78
	3. Percentage dry weight											
55	15.77	16.46	15.10	15.70	16.53	17.17	15.46	16.20	15.11	15.16	14.45	14.85
65	14.95	14.87	13.82	13.80	15.29	15.38	14.03	14.05	14.33	13.94	13.44	13.39
75	14.40	13.87	13.67	12.61	14.38	14.16	13.76	12.66	14.44	13.37	13.50	12.54
85	13.61	13.39	13.19	13.36	13.60	13.62	13.21	13.16	13.62	12.98	13.17	13.15

Differences required for significance at odds of 19 to 1:

1. Fresh weight:
   Leaves and stems 0.97
   Leaves 0.71
   Stems 0.38
2. Dry weight:
   Leaves and stems 0.16
   Leaves 0.13
   Stems 0.05
3. Percentage dry weight:
   Leaves and stems 0.42
   Leaves 0.64
   Stems 0.64

ing which assimilation predominated. Increase in dry weight with decrease in temperature during the dark period was probably caused by the lower respiration at low temperature. On the other hand, the

increase in dry weight with increase in temperature during the photoperiod from 55° to 75° F. probably reflected a net increase in assimilation with rising temperature.

Chemical composition

The carbohydrate and nitrogen metabolism of soybeans subjected to four constant temperatures and to combinations of four different temperatures during the light and dark periods has been determined. The plants were grown in the greenhouse for five weeks from time of planting. They were then divided into eight lots of sixty plants each and transferred to the control rooms previously described. Four lots were held at a constant temperature for the duration of the experiment. The other four lots were moved from one room to another at the end and the beginning of each photoperiod, so that they received different temperatures during light and dark periods. Temperature combinations during the light and dark periods were respectively 85°–55°, 75°–65°, 65°–75°, and 55°–85°. Daily photoperiods of 8 hours were applied for a period of five days. The plants were transferred from the greenhouse to the control rooms at the beginning of the photoperiod. In order to facilitate sampling, the lots of plants receiving constant temperature were moved into the control rooms one day and the lots receiving different temperatures during the light and dark periods the following day. Samples for chemical analyses were taken from representative lots each time before transferring them to the control rooms.

At the end of the dark period following the fifth short photoperiod the plants were harvested. The leaves were removed from the stems and samples for chemical analyses were prepared immediately. The method of sampling and the chemical methods employed for analyses have been reported previously (**10**). The only deviation was the method of determining the amount of cuprous oxide resulting from carbohydrate determinations. In these experiments the official volumetric permanganate method (**8**) was employed instead of the direct weight method. Results of the analyses are shown in tables 5, 6, and 7. Analyses of the two initial samples were very similar, indicating that the experimental lots were sufficiently homogeneous for the purpose of the experiment. Since intercomparison of the final com-

position of the various lots rather than net changes from beginning to end of the treatment was desired, results from these initial samples are not included in the tables. The percentage of dry weight (table 5) in the plants receiving constant temperature was greatest in the lot held at 55° F.; in those receiving alternating temperatures, greatest dry weight accumulation occurred in those receiving 55° during the dark period. Thus 16 hours of low temperature during the dark period decreased hydration of the tissue regardless of the temperature during the photoperiod.

TABLE 5

PERCENTAGE OF MOISTURE AND DRY WEIGHT AND FRESH AND DRY WEIGHT PER PLANT OF LEAVES AND STEMS OF SOYBEANS SUBJECTED TO VARIOUS TEMPERATURES DURING INDUCTION PERIOD OF FIVE 8-HOUR PHOTOPERIODS

Temperature (°F.)		Percentage moisture		Percentage dry weight		Fresh weight per plant after induction (gm.)			Dry weight per plant after induction (gm.)		
Photoperiod	Dark period	Leaves	Stems	Leaves	Stems	Leaves & stems	Leaves	Stems	Leaves & stems	Leaves	Stems
85°	85°	87.71	89.25	12.29	10.75	15.96	9.15	6.81	1.85	1.12	0.73
75	75	87.86	89.80	12.14	10.20	17.95	10.80	7.15	2.04	1.31	0.73
65	65	87.05	89.07	12.95	10.93	15.86	9.86	6.00	1.93	1.28	0.65
55	55	86.47	87.86	13.53	12.14	15.16	9.53	5.63	1.97	1.29	0.68
55	85	87.90	89.79	12.10	10.21	16.00	9.39	6.61	1.81	1.13	0.67
65	75	87.70	89.19	12.30	10.81	17.48	10.87	6.61	2.05	1.34	0.71
75	65	87.08	88.95	12.92	11.05	17.76	11.00	6.76	2.17	1.42	0.75
85	55	85.68	87.78	14.32	12.22	15.76	9.78	5.98	2.13	1.40	0.73

The plants that produced the greatest fresh weight (table 5) received a 75° F. temperature continuously, or during either the light or dark period. This increase in weight over the others was due primarily to an increase in the weight of the leaves. The dry weights (table 5) of the leaves and stems held at 85° during the dark period were less than those held at 55°. In the lots held at either 55° or 85° during the dark period an increase in temperature during the photoperiod resulted in an increase in dry weight. This agrees with the results shown in table 3.

The effect of temperature during the 5-day induction period on the distribution of nitrogen is shown in table 6. The percentage of

total nitrogen in the leaves and stems decreased as the temperature during the dark period decreased, regardless of the temperature during the photoperiod, and the percentage of soluble non-protein nitrogen showed the same relationship to temperature change. The percentage of ammonia nitrogen was approximately the same for all lots of leaves receiving constant temperatures. In those lots receiving different temperatures during the light and dark periods, the ammonia nitrogen in the leaves was higher in the lots receiving low temperatures during the photoperiod. In the stems the percentage

TABLE 6

NITROGEN DISTRIBUTION IN LEAVES AND STEMS OF SOYBEANS SUBJECTED TO VARIOUS TEMPERATURES DURING INDUCTION TREATMENT OF FIVE 8-HOUR PHOTOPERIODS

Temperature (°F.)		Total nitrogen		Soluble non-protein nitrogen		Ammonia nitrogen		Nitrate nitrogen	
Photoperiod	Dark period	Leaves	Stems	Leaves	Stems	Leaves	Stems	Leaves	Stems
		Percentage dry weight							
85°	85°...	4.736	2.322	1.326	0.986	0.008	0.037	0.089	0.223
75	75....	4.697	2.148	0.964	0.853	0.008	0.009	0.074	0.235
65	65....	4.081	1.716	0.865	0.677	0.007	0.009	0.038	0.155
55	55....	3.965	1.660	0.776	0.724	0.007	0.008	0.044	0.156
55	85....	4.549	2.501	1.265	1.529	0.024	0.029	0.090	0.225
65	75....	4.173	1.986	0.862	1.009	0.032	0.027	0.032	0.166
75	65....	4.092	1.787	0.797	0.824	0.015	0.018	0.038	0.153
85	55....	3.662	1.850	0.642	0.589	0.013	0.024	0.020	0.081
		Milligrams per plant							
85	85....	53.25	16.95	14.91	7.21	0.09	0.27	1.00	1.63
75	75....	61.56	15.65	12.63	6.22	0.10	0.07	0.97	1.71
65	65....	52.06	11.22	11.04	4.44	0.09	0.06	0.49	1.02
55	55....	51.08	11.31	10.00	4.95	0.09	0.05	0.57	1.06
55	85....	51.64	16.85	14.36	10.31	0.28	0.19	1.03	1.52
65	75....	55.76	14.14	11.52	7.20	0.43	0.19	0.43	1.18
75	65....	58.08	13.31	11.33	6.15	0.22	0.13	0.55	1.14
85	55....	51.24	13.45	8.99	4.30	0.19	0.17	0.29	0.59

of ammonia was uniform in all lots receiving constant temperature, except the 85°–85° lot, which was much higher than the others. In the other four lots there was little difference in the percentage of

TABLE 7

CARBOHYDRATE CONTENT OF LEAVES AND STEMS OF SOYBEANS SUBJECTED TO VARIOUS TEMPERATURES DURING INDUCTION TREATMENT OF FIVE 8-HOUR PHOTOPERIODS

TEMPERATURE (°F.)		REDUCING SUGARS		TOTAL SUGARS		SUCROSE		STARCH	
PHOTOPERIOD	DARK PERIOD	LEAVES	STEMS	LEAVES	STEMS	LEAVES	STEMS	LEAVES	STEMS
		PERCENTAGE DRY WEIGHT							
85°	85°...	0.805	0.940	1.773	1.554	0.805	0.465	1.196	0.689
75	75....	1.079	1.511	2.061	2.109	0.816	0.451	1.302	0.755
65	65....	2.047	4.290	2.998	4.967	0.765	0.457	3.454	1.271
55	55....	3.854	7.471	4.907	8.459	0.820	0.675	3.362	2.594
55	85....	0.810	1.009	0.934	1.490	0.016	0.352	1.215	1.107
65	75....	1.253	1.685	2.017	2.481	0.602	0.648	1.765	1.212
75	65....	1.772	3.722	2.414	4.556	0.464	0.615	2.306	1.485
85	55....	2.960	5.978	3.889	7.272	0.733	1.031	4.301	2.481
		MILLIGRAMS PER PLANT							
85	85....	9.05	6.87	19.94	11.37	9.05	3.40	13.45	5.03
75	75....	14.14	11.01	27.00	15.37	10.69	3.28	17.06	5.50
65	65....	26.12	28.14	38.35	32.58	9.76	3.00	44.07	8.34
55	55....	49.65	51.06	63.27	57.82	10.57	4.61	43.36	17.73
55	85....	9.20	6.80	10.61	10.04	0.18	2.37	13.80	7.46
65	75....	16.73	12.03	26.95	17.71	8.04	4.62	23.58	8.65
75	65....	25.19	27.78	34.32	34.00	6.60	4.59	32.78	11.08
85	55....	41.46	43.65	54.47	53.10	10.26	7.53	60.24	18.11

ammonia. The percentage of nitrate nitrogen in the leaves decreased as the temperature during the dark period was lowered, regardless of the temperature during the photoperiod. In the stems the nitrate nitrogen was higher in the 85° and 75° constant temperature lots than in the 65° and 55° lots. In the remaining four lots nitrates decreased as the temperature during the dark period decreased. All the organic and inorganic nitrogen fractions determined showed a progressive

decrease as the temperature during the dark period was lowered. While this general and uniform trend invites speculation concerning the effect of temperature on nitrogen metabolism, it is advisable to obtain more data as to the condition of the plants at the end of the light period as well as at the end of the dark period before making any generalization.

When the results were expressed as milligrams per plant the relationships were different since the size of the plant entered into the calculations. The maximum amount of total nitrogen occurred in the leaves of the lot of plants held at 75° continuously. The leaves of the two lots of plants held at 55°–55° and 55°–85° contained the same milligrams of total nitrogen, but these plants were very different in their growth habits. The former were short and sturdy while the latter were much elongated. In the stems the milligrams of total nitrogen decreased as the temperature during the dark period decreased. The actual milligrams of soluble non-protein nitrogen, ammonia, and nitrate nitrogen in both leaves and stems varied in the same manner as the percentage figures previously discussed.

When the soluble non-protein nitrogen was calculated as a percentage of the total nitrogen, the percentage in the leaves decreased as the temperature during the dark period decreased. In the stems the percentage was not very different in any of the lots when the temperature remained constant, but when the temperature was different during the light and dark periods the percentage decreased with the lower temperatures during the dark period.

The results obtained on the carbohydrate fractions are shown in table 7. The reducing sugars present in the leaves and stems were greatly influenced by the temperature during the dark period. The amount in these tissues nearly doubled with each 10° drop. The total sugar also increased as the temperature of the dark period decreased, but the magnitude of increase in the leaves was not as great for each 10° drop as it was in the stems. The amount of sucrose in the leaves was approximately the same in all the lots held at a constant temperature. When the temperatures during light and dark periods were varied, sucrose was most abundant in the lot receiving a 55° temperature during the dark, and least abundant at 85°. In the stems the amount of sucrose was high in the lots receiving a 55° dark

period, regardless of the temperature during the photoperiod; but the lot receiving an 85° photoperiod contained more sucrose than the one receiving 55° continuously. The percentage of starch in the leaves and stems increased as the temperature of the dark period decreased. The two lots of plants held continuously at 85° and 75° contained approximately the same amount of starch, and the same was true of the lots held at 65° and 55°. The former two lots, however, were much lower in starch than the latter two. In the lots that received different temperatures during light and dark there was a progressive increase in starch in both leaves and stems as the temperature during the dark period decreased. These data indicate that the plants that received a 55° dark period, regardless of the temperature during the photoperiod, were prepared to start their next photoperiod with a high concentration of carbohydrates in both leaves and stems, while the plants receiving 85° during the dark had a very much lower concentration of carbohydrates at this time. The 75° and 65° lots were intermediate to these two extremes. Thus during induction treatments the different lots attained entirely different carbohydrate composition.

The carbohydrate and nitrogen data in tables 6 and 7 show directly opposite trends with change in temperature. When the total carbohydrates were low the soluble non-protein nitrogen was high, and when total carbohydrates were higher the soluble non-protein nitrogen was low. A low temperature during the dark period resulted in carbohydrate accumulation and a decrease in soluble non-protein nitrogen, while a high temperature resulted in a reverse relationship. Consequently these lots had extremely different carbohydrate and nitrogen relationships at the termination of their 5-day induction period.

Experimental results with 16-hour photoperiod

The data thus far presented have shown that temperature differences applied during induction with 8-hour photoperiod bring about differences in number of flower primordia initiated. The question arises as to whether any of the temperature conditions alone might cause floral initiation even though the daily photoperiod during treatment was kept at 16 hours. To determine this point an experi-

ment was set up similar to that reported in table 1, in so far as temperature treatments were concerned, but differing in that 16-hour photoperiods were used. In this experiment half the plants were subjected to differential temperature treatments for five days and the remainder for ten, and were then returned to the greenhouse. They were harvested and dissected three weeks after the beginning of the treatments. In 480 experimental plants four were found that had initiated flower primordia. These were not associated with any particular treatment but were distributed at random among the lots, indicating that factors other than temperature were operating. The probable cause of initiation in these four cases was age of plant. They were nearly nine weeks old at the time of dissection, and it has been previously shown (10) that initiation of flower primordia on Biloxi soybeans takes place even on long photoperiod when the plants become older. The results show that the temperature treatments applied in these experiments were ineffective in causing initiation of flowers when the plants received long photoperiods during treatment.

An experiment was also conducted on 16-hour photoperiod to determine the differences in fresh and dry weight and dry weight percentages (table 4). The experimental conditions were the same as those of the experiment reported in table 3, except that a 16-hour instead of an 8-hour photoperiod was employed during the 5-day treatment.

Differences in both fresh and dry weight that were statistically significant were associated with variations in temperature of both the light and dark periods. Significant differences in percentage of dry weight, however, occurred only in response to variations in temperature during the dark period. The highest dry weight percentages were associated with the lowest temperatures, and as the temperature increased the dry weight percentages became less.

In general the fresh and dry weight data in the experiments on 8-hour and 16-hour photoperiod showed similar variations with corresponding treatments. A higher percentage of dry weight and more dry weight per plant resulted from 16-hour than from 8-hour photoperiods. This difference was to be expected since the former group

received twice as much total radiation as the latter, and the difference does not seem to have any relation to the extent of floral initiation in the two groups.

Discussion

Temperature alone, under the conditions of these experiments, was not effective in causing floral initiation. Plants held on 16-hour photoperiod remained vegetative regardless of the combinations of temperature applied during the light and dark periods. When similar plants were subjected to 8-hour photoperiods during the application of the temperature treatments, flower buds were initiated in nearly all lots. The number of plants per lot initiating flower buds, however, and also the number of such buds formed per plant, varied considerably from one treatment to another.

It has been shown that significant differences in total nodes per plant existed among the different lots at the end of the induction treatment. The trend of these differences was similar to the trend of the differences observed in floral initiation, but their magnitude was much less. The plants of the lot kept at 75° during the photoperiod and 65° during the dark period, for example, had only 0.5 more nodes per plant than those kept at 75° during the photoperiod and 55° during the dark period, but they initiated flower primordia in 2.1 more buds per plant.

The plants at the higher temperature during the dark period were evidently initiating new nodes at a slightly greater rate than those at the lower temperature, and therefore it was expected that they would likewise be slightly more active in floral initiation. The actual increase, however, in number of plants forming flower buds and in number formed per plant at the higher temperature was far in excess of what would be expected from this cause alone.

In table 1 it was also shown that increases in temperature during the dark period from 55° to 65° and from 65° to 75° resulted in approximately equal increases in the average number of nodes formed per plant. The first 10° rise in temperature, however, resulted in a tremendously greater increase in floral initiation than the second. While the actual rate of differentiation of new structures at the growing points during induction treatments may be an important factor

in determining the effectiveness of such treatment, it seems evident from the data that other factors play an important role.

The great increase in floral initiation induced by a change in temperature during the dark period from 55° to 65° points strongly to the importance of some type of reaction occurring during this period that makes possible the initiation of flower primordia. It does not seem possible to account for the great differences in floral initiation occurring with changes in temperature during the dark period on the basis of changes in the carbohydrate and nitrogen composition. While changes of considerable magnitude were found in these fractions, their trends were unlike those found in the morphological data. In general the carbohydrates decreased and the nitrogen fractions increased with rise in temperature during the dark period. Initiation of flower primordia attained a maximum at 65° and 75° during the dark period and decreased slightly at 85°. Thus the changes in chemical composition were progressive with each 10° rise in temperature, while the morphological responses rose to a maximum and then decreased.

While changes in carbohydrate and nitrogen fractions do not seem to explain the increase in floral initiation with increase in temperature from 55° to 65° during the dark period, some chemical reaction, the nature of which is unknown, must be responsible for these increases. As measured by the floral response of the plants, the activity of such a reaction was limited at a temperature of approximately 55° during the dark period. Its activity increased sharply with increase in temperature during the dark period, the increase in the first 10° rise being very much greater than in the second. The smaller increase in activity during the second 10° rise may have been associated with the fact that the rate of differentiation of new structures by the plant imposed a limit on the rate at which flower primordia were formed.

In Biloxi soybean, conditions during the photoperiod and during the dark period can both exert a controlling influence upon floral initiation. In previous work (1) it was shown that if the light intensity was low, initiation did not occur regardless of the photoperiod employed. When intensity was high, however, initiation oc-

curred only when the photoperiod was short, indicating the necessity of the dark period. In this investigation it is further shown that when light intensity during the photoperiod is adequate, great variations in the extent of floral initiation may be induced by variations in temperature during the dark period.

Summary

1. Biloxi soybeans were grown in the greenhouse for four to five weeks and transferred to a series of control rooms where various combinations of temperature during the photoperiod and the dark period were applied for five days. Photoperiods of both 8 and 16 hours were used.

2. The effect of these various temperatures on the initiation of flower primordia has been determined.

3. Initiation of flower primordia was influenced to a much greater extent by variation in temperature during the dark period than by variation during the photoperiod.

4. When the temperature during the photoperiod was constant, a 55° temperature during the dark period limited the amount of initiation that occurred. At 65° initiation was much more extensive. This rise of 10° in temperature during the dark period produced a greater difference in initiation than any other temperature variation of the same amount.

5. Variation in temperature resulted in the formation of different numbers of nodes. These differences, however, were not great enough to account for the differences in floral initiation.

6. Other measurements, such as fresh and dry weights, stem lengths, and leaf areas, were made. All were influenced by the different temperature treatments.

7. The carbohydrate and nitrogen content of the plants was also influenced greatly by the different temperature treatments. As the temperature during the dark period was increased the carbohydrates decreased and the nitrogen fractions increased. These changes in carbohydrate and nitrogen metabolism could not be correlated with the morphological responses.

8. None of the temperature combinations used in these experiments brought about the initiation of flower primordia when photoperiods of 16 hours were used.

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PHOTOPERIODIC INDUCTION AS INFLUENCED BY ENVIRONMENTAL FACTORS[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 504

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(WITH TWO FIGURES)

Introduction

Xanthium pennsylvanicum may be photoperiodically induced by exposure to a single dark period of 15 hours at 70° F., even though subsequently grown on photoperiods not directly conducive to floral initiation. Since several lines of evidence (**5**) indicate that photoperiodic induction is determined by length of the dark period, it is perhaps more appropriate, with this plant, to apply the term "critical dark period" rather than "critical day" or "critical photoperiod," as has been applied to some other plants (**4**). Length of the critical dark period for *Xanthium* is $8\frac{1}{2}$ to 11 hours, depending upon environmental factors, of which temperature seems to be the most effective.

It has been demonstrated (**1, 2, 3, 5, 6, 7, 8, 9, 10, 11**) that the leaves of several species of plants, including *Xanthium*, perceive the photoperiodic stimulus. Whether the leaves are the locus of photoperiodic induction is not known, but indications from the present work are that this is the case. Since the leaves perceive the photoperiodic stimulus, and since usually the stimulation occurs with exposure to 9 hours of darkness and not 8 hours, an attempt was made to determine what environmental factors influence the length of dark period necessary for induction. This seemed particularly pertinent since *Xanthium* during the winter months may respond variously, sometimes a particular treatment resulting in flowering and at other times not. The environmental factors which obviously change considerably from summer to winter in the greenhouses at Chicago are light intensity, temperature, and humidity; therefore the effect of variation in these factors upon length of the critical dark period was investigated.

[1] This investigation was aided in part by a grant to the University of Chicago from the Rockefeller Foundation.

General methods

The cocklebur, *Xanthium pennsylvanicum*, was used for most of these experiments. Plants were grown from seeds collected in the vicinity of Chicago in November. Until desired for use, the fruits were stored in a bin out of doors, exposed to variations of weather. The seeds were then removed from the fruits, scratched slightly to rupture the coats, and planted within a few days in a light garden soil in ordinary flats. The seedlings were transplanted singly to pots when they had attained heights of 4–6 inches. Before the seeds germinated, the flats were placed on a well lighted bench in the greenhouse where supplementary illumination was provided by Mazda light of approximately 100 foot candles at the soil surface from sundown until 2:00 A.M. (For convenience, these conditions are referred to here as the long photoperiod.) This photoperiod varied considerably during the course of the work, but was never less than 18 hours in duration. *Xanthium* plants kept continuously under these conditions remained strictly vegetative, some for nearly a year, showing no sign of flower initiation when dissected. Biloxi soybeans were also used as experimental material. The seeds were planted in fertile soil at the rate of four per 5-inch clay pot. The pots were placed on long photoperiod and watered regularly until used for experiment.

Excessively high temperatures and low humidities were minimized by careful ventilation of the greenhouse and frequent sprinkling of the walls and benches. The plants were watered frequently with tap water, and under optimum conditions for growth, as often as twice weekly with a double strength of Shive's (12) R_2S_5 nutrient solution. Unless otherwise stated, the plants grew vigorously throughout the experimental period. In general, after a given treatment the plants were returned to long photoperiod and allowed to grow until harvested.

Except when clearly evident macroscopic flowers developed, flowering response of cockleburs and soybeans was determined by careful dissection under a binocular microscope with a magnification of 20.7. Usually only the terminal buds of cocklebur were dissected, as in that species the terminal bud is almost invariably the first to initiate floral primordia, and therefore is considered the most depend-

able one in determining flowering response. With soybean it was necessary to dissect all the buds, for the terminal meristem produces floral parts only after a number of days of treatment (1), while in the vicinity of the fifth node from the terminal meristem they are differentiated 3–5 days after first being subjected to short photoperiod.

Investigation

Residual effect in leaf resulting from photoperiodic treatment

Various experiments have shown (5) that a single photoperiodically induced intact leaf of *Xanthium* may effect floral initiation in a

FIG. 1.—*A*, induced leaf grafted to vegetative plant, 4 weeks after grafting. As a result of the graft the plant has initiated floral primordia. *B*, enlarged view of grafted leaf.

non-induced plant when the plant is grafted to the one bearing the induced leaf. It might appear that such a leaf, after having been induced, could be removed from the plant on which it was borne and

be transferred to a vegetative non-induced plant with the result that the vegetative plant would flower because of a residual stimulus or substance transmitted to it from the leaf grafted upon it. To test this possibility, six *Xanthium* plants were subjected to two consecutive 15-hour dark periods separated by a photoperiod during which induction was completed. During the photoperiod following the second long dark period, two leaves from each of the treated plants were removed by severing the petiole obliquely across the axis about 0.5 inch from the stem. Two leaves of each of six vegetative plants which had been maintained on long photoperiod were similarly removed and the leaves from the induced plants grafted to the vegetative ones, all being subsequently maintained on long photoperiod.

TABLE 1

EFFECT OF GRAFTING LEAVES OF INDUCED PLANTS TO VEGETATIVE PLANTS

TREATMENT	NO. OF PLANTS	FLOWERING CONDITION AFTER FOUR WEEKS
Two leaves from photoperiodically induced plants grafted to each vegetative plant....	6	3 with flower primordia 3 vegetative
Two leaves from vegetative plants grafted to each vegetative plant (controls).....	6	6 vegetative

The contact was accomplished by placing one end of a short piece of glass tubing over the cut stump of the petiole of the vegetative plant and inserting the cut end of the leaf into the other end of the tube in such a way that the two cut surfaces were in intimate contact (fig. 1). The glass tube served to support the leaf and hold the cut surfaces in close contact. The plant was then placed in a nearly saturated atmosphere in a glass chamber and exposed to long photoperiod. To serve as controls for these six experimental plants, leaves from six vegetative plants were removed and grafted to six other vegetative plants in a similar manner. These control plants were maintained by the side of the experimental plants. The results are shown in table 1.

Although only three of the treated plants initiated flowers, these were the ones on which the grafted leaves remained alive for practically the entire period of the experiment (fig. 1). Most of the grafted leaves died within 10 days after grafting.

Leaves may be photoperiodically induced and subsequently may supply substances leading to floral initiation under conditions of long photoperiod or in whole plants no part of which has undergone any other type of inductive treatment.

Residual effect of treatment with short photoperiod insufficient to bring about photoperiodic induction

In the subsequent discussion relating to subjection of plants to dark periods of varying length, unless otherwise stated a long dark period is one of 15 hours accompanied by a 9-hour photoperiod, and a short dark period one of 8 hours accompanied by a 15-hour photoperiod. Although *Xanthium* may be induced to flower when subjected to a single dark period at 70° F., as many as eight long dark periods may be required if the temperature during the dark period is 40° F. Biloxi soybean may initiate flower primordia as the result of exposure to three long dark periods alternating with short photoperiods at 70°. When more than a single long dark period is required for induction, it is possible that there may be a residual effect of each of the dark periods, which being cumulative finally results in the induced condition. In order to test such a possibility the following experiments were carried out.

On April 1, 192 vegetative *Xanthium* plants were selected for uniformity and exposed to two long dark periods at 40° F., the two periods being separated by a short photoperiod in the greenhouse at 70°. They were then returned to long photoperiod at 70°. Sixty more were selected to serve as initial controls and divided into ten lots of six plants each. One lot was exposed to one long dark period at 40° F., one lot to two long dark periods at 40°, and so on, the last lot receiving a total of ten long dark periods at 40°, each dark period being separated by a short photoperiod at 70° as previously indicated. After final treatment each lot of these initial controls was returned to the greenhouse to continue on long photoperiod until the entire experiment was harvested. Fifty other plants, selected at the same time as final controls, were divided into ten lots of five plants each and treated exactly as were the initial controls except that their

long dark period ran concurrently with that of the last group of the experimental plants.

The original 192 plants, all of which had received two long dark periods at 40° F. when returned to the long photoperiod bench, were divided into four groups of forty-eight plants each. One of these groups remained on long photoperiod for 2 days before further treat-

TABLE 2

FLOWERING RESPONSE OF XANTHIUM PLANTS TREATED WITH VARIOUS NUMBERS OF 15-HOUR DARK PERIODS AT 40° F.*

TREATMENT RECEIVED PREVIOUS TO EXPOSURE TO 15-HOUR DARK PERIODS	DATES OF LONG DARK PERIOD TREATMENTS	NO. OF 15-HOUR DARK PERIODS (AT 40°) RECEIVED SUBSEQUENT TO TREATMENT									
		1	2	3	4	5	6	7	8	9	10
No previous treatment (initial controls).....	April 4–13	6 veg	6 veg	6 veg	6 veg	6 veg	5 veg 1 fl	6 veg	6 veg	5 veg 1 fl	2 veg
Two long dark periods at 40°, then 2 short dark periods in greenhouse...	April 1 & 2; April 5–14	6 veg	6 veg	6 veg	6 veg	6 veg	6 veg	6 veg	6 veg	6 veg	6 veg
Two long dark periods at 40°, then 4 short dark periods in greenhouse...	April 1 & 2; April 7–16	6 veg	6 veg	6 veg	6 veg	6 veg	6 veg	.....	2 veg 4 fl	.....	1 veg 4 fl
Two long dark periods at 40°, then 8 short dark periods in greenhouse...	April 1 & 2; April 11–20	6 veg	6 veg	6 veg	6 veg	6 veg	5 veg 1 fl	.....	2 veg 4 fl	.....	..... 6 fl
Two long dark periods at 40°, then 16 short dark periods in greenhouse...	April 1 & 2; April 14–23	6 veg	6 veg	6 veg	6 veg	6 veg	5 veg 1 fl	.....	5 veg 1 fl	.....	6 veg
No previous treatment, but maintained on short dark periods until April 14 (final controls).....	April 14–23	5 veg	5 veg	5 veg	5 veg	5 veg	4 veg 1 fl	2 veg 3 fl	3 veg 2 fl	3 veg 2 fl	2 veg 3 fl

* All photoperiods and all dark periods (other than those at 40°) occur in the greenhouse.

ment, a second group remained for 4 days, a third for 8 days, and the fourth for 16 days. Each group was then subdivided into eight lots of six plants each, one lot being exposed to one long dark period at 40° F., one to two long dark periods, one to three, one to four, one to five, one to six, one to eight, and one to ten (table 2).

Although growing conditions in March, just previous to beginning the experiment, had been reasonably favorable for rapid growth, the weather from April 1 to April 20 was very cloudy. It is possible that the variable results are owing to variation in light intensity during the experimental period.

While no definite conclusions could be drawn, indications were that there was not sufficient residual effect of a long dark period at 40° in *Xanthium* to persist through two or more long photoperiods at 70°.

Another experiment was designed to show whether there is any residual effect at all, and if so, over how many long photoperiods it persists. For this experiment, beginning April 26, thirty-six pots of *Xanthium* plants were given an initial treatment of four consecutive

TABLE 3

RESPONSE OF XANTHIUM PLANTS TO INITIAL TREATMENT OF FOUR LONG DARK PERIODS AT 40° FOLLOWED BY EIGHT ADDITIONAL LONG DARK PERIODS EACH INTERSPERSED WITH VARYING NUMBERS OF SHORT DARK PERIODS AT 70° F.

No. of long dark periods of initial treatment	No. of short dark periods intervening between additional long dark periods	No. of plants with flower primordia	No. vegetative
Four	1	5	1
	2	0	6
	3	0	6
	4	0	6
	5	0	6
Four (controls)	(on continuous long photoperiod after initial treatment)	1	5

15-hour dark periods at 40°. Thereafter, six pots were returned to continuous long photoperiod as controls. Another group of six pots received, after the initial treatment, alternate short dark periods at 70° and long dark periods at 40° until they had had a total of twelve long dark periods. A third group of six got two short dark periods, a long dark period, two short dark periods, a long one, etc., until they had had a total of twelve long dark periods. The fourth, fifth, and sixth groups of six were treated in the same way except that the period of short dark period treatment was progressively one dark period longer in each. Results of this experiment (table 3) indicate that there is some cumulative effect of the long dark periods, but that it diminishes rapidly under the influence of short dark periods.

Those plants which received four long dark periods at 40°, followed by alternating short dark periods at 70° and long dark periods at 40°, produced flowers. On the other hand, none of those plants whose long dark period treatment was interrupted periodically by exposures to two or more short dark periods showed any indication of flowering. Indications are that, in *Xanthium*, a treatment with four long dark periods at 40° produces an effect in the plant, such an effect being carried over for more than 24 hours.

It might be assumed that one dark period at 40° would cause flowering if the dark period were of sufficient length. That this is very unlikely is shown by the following experiment. Forty pots of *Xanthium* were placed in the darkroom at 40° on May 9, and at more or less regular intervals four pots were removed and placed on long photoperiod in the greenhouse. None of the plants flowered even though some of them received 112 hours of continuous darkness. All plants seemed in excellent condition when removed from the darkroom. Apparently, under these conditions of low dark period temperature, photoperiodic induction does not take place unless the long dark periods are accompanied by short photoperiods.

Experiments with Biloxi soybeans

Experiments similar to those just reported were performed with soybeans. Since for induction, soybeans ordinarily require at least three long dark periods at greenhouse temperatures, it was not necessary to use the refrigerated room for dark period treatments.

On March 22, ninety-six pots, each containing four vegetative Biloxi soybean plants, were selected for uniformity from those growing on the long photoperiod bench. Excepting twenty-four pots reserved for controls, half the plants were given an initial treatment of one 15-hour dark period and half an initial treatment of two 15-hour dark periods. After the initial treatment the plants were removed to the long photoperiod bench, one-third of each group remaining there for 5 days, one-third for 10, and one-third for 20 days. After receiving the specified number of long photoperiods, one-fourth of each group received one additional long dark period; one-fourth, two long dark periods; one-fourth, three long dark periods; and one-fourth, five long dark periods. The twenty-four pots of controls were divid-

ed into two groups to serve as initial and final controls. Beginning March 22, two pots of initial controls were given one long dark period; two pots two consecutive long dark periods; two pots, three; two, four; two, five; and two, seven. The final controls remained on the long photoperiod bench continuously until April 13, when they began a course of treatment similar to that of the initial controls.

After the treatments with long dark periods, all plants were returned to the long photoperiod bench where they remained for 2 weeks, after which they were carefully dissected to determine whether flowers or flower primordia were present (table 4).

TABLE 4

FLOWERING RESPONSE OF BILOXI SOYBEANS EXPOSED TO EITHER ONE OR TWO PERIODS OF LONG DARK PERIOD TREATMENT AT DIFFERENT STAGES OF GROWTH*

No. of long dark periods of exposure in initial treatment	No. of short dark periods intervening between initial and final treatments	Date of beginning of final treatment	No. of long dark periods of final treatment					
			1	2	3	4	5	7
Initial controls	None	March 22	8 veg 0 fl	7 veg 0 fl	5 veg 2 fl	2 veg 5 fl	..... 7 fl	..... 7 fl
One long dark period on March 22	5	March 28	7 veg	8 veg	8 veg	.....	6 fl	.....
	10	April 2	8 veg	8 veg	6 veg 2 fl	.....	7 fl 7 fl	....
	20	April 12	7 veg	8 veg	7 veg	.....	..... 8 fl	....
Two long dark periods on March 22 and 23	5	March 29	7 veg	7 veg	6 veg 1 fl	.....	2 veg 5 fl	.....
	10	April 3	8 veg	7 veg	8 veg	.....	..... 7 fl	....
	20	April 13	6 veg 1 fl	7 veg 1 fl	4 veg 4 fl	....	1 veg 6 fl	....
Final controls	None	April 13	8 veg	7 veg 1 fl	7 veg 1 fl	1 veg 7 fl	..... 8 fl	..... 8 fl

* Plants grown on long photoperiod until March 22, when all were vegetative. After the various treatments all buds were carefully dissected and presence or absence of flower primordia noted. If a plant possessed one or more flower primordia it is reported as flowering (fl); other plants reported as vegetative (veg).

As the plants grew older they required fewer long dark periods for induction. Thus the initial controls, treated March 22, showed some flowering 3 days after treatment and all flowered after 5 days. The final controls, treated April 13, showed some flowering after treatment for 2 days and all but one flowered after 4 days. This indicates that as the plants grow older they become more sensitive to photoperiodic treatment. The results show that the effect of an initial

TABLE 5

RESPONSE OF BILOXI SOYBEANS TO INITIAL TREATMENT OF TWO LONG DARK PERIODS FOLLOWED BY EIGHT LONG DARK PERIODS EACH INTERSPERSED WITH VARYING NUMBERS OF SHORT DARK PERIODS

No. of long dark periods of initial treatment	No. of short dark periods intervening between additional long dark periods	No. of plants with flower primordia	No. vegetative
Two	1	0	12
	2	3*	13
	3	0	14
	4	0	10
	5	0	14
Controls	(on continuous long photoperiod after initial treatment)	0	6
Two		1	5
Three		5	2
Four		5	1
Five			

* It is possible that these plants were shaded sufficiently by others so that their supplementary illumination on the long dry bench was too low to prevent flowering.

treatment of one or two long dark periods does not persist over as many as five long photoperiods.

Another experiment was carried out to determine the possible residual effect over fewer long photoperiods. For this, twenty-eight pots were selected. Of the eight pots of controls, two received two consecutive long dark periods; two, three long dark periods; two, four; and two, five before being returned to the long photoperiod bench. The other twenty pots all received an initial treatment of two long dark periods, after which they were divided into five groups of four pots each. The first group then received a short dark period, a long dark period, a short one, and so on, until the plants had been

subjected to a total of ten long dark periods, when they were returned to the long photoperiod bench until dissected. The remaining four groups were treated in essentially the same manner except that the period of short dark periods between each of the single long dark periods of the final treatment was progressively one dark period longer for each group. The results of this experiment (table 5) unmistakably indicate that the effect of two long dark periods does not persist for more than 24 hours.

Residual effect of treatment with short photoperiod sufficient to bring about photoperiodic induction

Since these two experiments indicated that there was no appreciable residual effect of long dark period treatments too brief to bring about floral initiation, the question arose as to whether there was an appreciable residual effect provided the initial treatment was sufficient to cause floral initiation. In consequence, a third experiment was carried out.

On March 19, 150 pots each containing four vigorously growing, 1-month old, vegetative Biloxi soybean plants were selected for uniformity from 500 pots on the long photoperiod bench. The various treatments are given in table 6. Those plants treated with long dark periods during the period March 19–24 were placed under the diurnal photoperiod in the greenhouse at that time, without supplementary illumination. Subsequent to March 24 all plants, when exposed to long dark periods, were given dark periods of 15 hours at greenhouse temperatures.

The plants were harvested May 22, at which time eight uniform plants of the sixteen on each experimental treatment were carefully dissected and the presence of flowers at each node on the main axis noted according to the method outlined by Borthwick and Parker (1). As may be seen in table 6, those plants which received the greatest number of long dark periods had the greatest number of flowers.

Treatment with long dark period from the period March 19–30 was not so effective in bringing about floral initiation as were the later periods. This may be related to the fact that the plants were relatively young during the earlier period, or it may be that the long dark periods were not so effective because they were only the natural

night lengths of that time and thus were slightly shorter than 15 hours. It may also be noted that those plants which received two

TABLE 6

EFFECT OF VARIOUS SUCCESSIVE TREATMENTS WITH LONG DARK PERIOD UPON FLOWER BUD FORMATION IN BILOXI SOYBEANS

TREATMENT (PLANTS HARVESTED MAY 22)	NO. OF LONG DARK PERIODS	DATE	NO. OF PLANTS WITH FLOWER PRIMORDIA	TOTAL BUDS ON MAIN AXIS OF EIGHT PLANTS CONTAINING FLOWER PRIMORDIA
No long dark period until March 19, then:	1	March 19	0	0
	3	March 19–21	0	0
	5	March 19–23	7	11
	6	March 19–24	7	14
	7	March 19–25	8	22
	9	March 19–27	8	26
Six long dark periods*March 19–25, then ten long photoperiods, March 25–April 4, then:	1	April 4	7	13
	3	April 4–6	7	15
	5	April 4–8	8	28
	7	April 4–10	8	39
	9	April 4–12	8	59
No long dark period until April 1, then:	1	April 1	0	0
	3	April 1–3	0	0
	5	April 1–5	5	14
	7	April 1–7	8	31
	9	April 1–9	8	44
Six long dark periods*March 19–25, then twenty long photoperiods, March 25–April 14, then:	1	April 14	7	14
	3	April 14–16	6	15
	5	April 14–18	8	38
	7	April 14–20	8	42
	9	April 14–22	8	60
No long dark period until April 10, then:	1	April 10	0	0
	3	April 10–12	3	6
	5	April 10–14	8	25
	7	April 10–16	8	35
	9	April 10–18	8	41

* These long dark periods were equal to the duration of the natural night during this period and of approximately 13 hours; all other long dark periods were 15 hours.

treatments of long dark periods developed approximately as many flowers as the combined numbers produced on two separate sets of plants, one set receiving a number of long dark periods comparable

with the first treatment of the former plants and the second receiving a treatment comparable with the second treatment.

This relationship is shown more clearly in figure 2. Here three sets of plants are shown diagrammatically. Figure 2*A* shows each

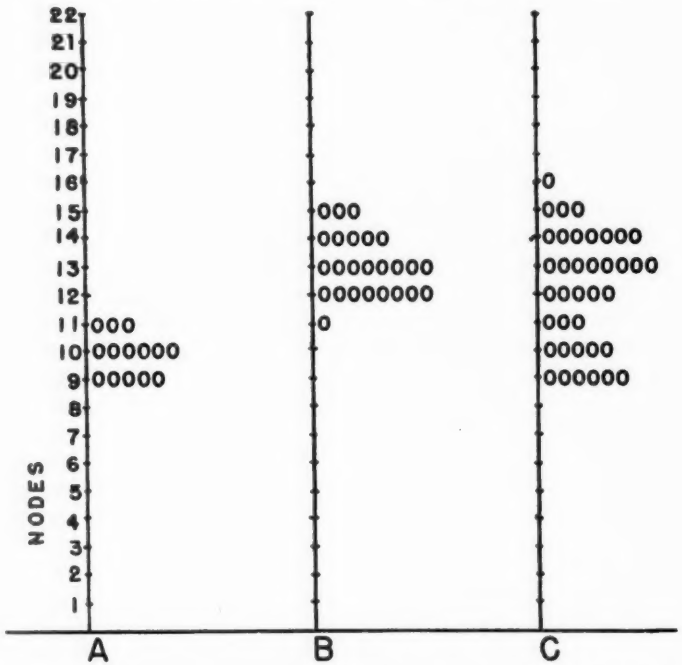

FIG. 2.—Diagrammatic representation of effect on Biloxi soybeans of various long dark period treatments.

flower of the eight plants which received six long dark periods (March 19–26). Each flower is shown in its relative position on the plant. Figure 2*B* illustrates the flowering condition of the eight plants which received five long dark periods (April 10–15). Figure 2*C* illustrates those plants which received both long dark period treatments; that is, six long dark periods (March 19–26) and five long dark periods (April 10–15). It may be seen that *C* represents almost precisely a composite picture of *A* and *B*.

These results all indicate that in soybean an induction period of several long dark periods acts more or less independently of previous treatments if the various treatments are separated by one or more short dark periods.

This is somewhat in contrast to *Xanthium* plants which are exposed to long dark periods at 40° F. In *Xanthium* the residual effect lasted 24 hours or more. In soybean also, even though a plant receives an induction period of sufficient length to bring about floral initiation, in order that more flowers be produced by a subsequent photoperiodic treatment the plant must undergo a second induction period. This is again in contrast to *Xanthium* which, once it is photoperiodically induced, seems to remain more or less permanently so and will continue to produce additional primordia over a long period of time.

Effect of temperature on critical dark period length of Xanthium plants of two different ages

Since a low dark period temperature produces a profound effect upon the length of time required for induction of *Xanthium* (**5**), it was decided to ascertain just what effect temperature exercised upon the critical photoperiod. Results have repeatedly been obtained in this work, and in that of Hamner and Bonner (**5**), which indicate that the critical dark period is between 8 and 9 hours with summer growing conditions and dark period temperatures between 70° and 80° F. In the following experiment a determination was made of the critical dark period of plants exposed to photoperiods in the greenhouse and dark periods at 40° F.

Two hundred and forty vegetative plants were selected from among those growing on the long photoperiod bench. One-half were approximately 3 weeks old and about 6 inches in height, possessing three to five fully expanded leaves. The other half were a month older, about 1 foot in height, possessing eight to eleven fully expanded leaves. Each of these two groups was divided into twenty-four lots containing five plants each. Each lot of five young plants was combined with a corresponding lot of older plants, making twenty-five lots each of ten plants for each experimental treatment. Sixteen of these lots then received a treatment of fifteen consecutive dark

periods at 40° F. The first lot was subjected to dark periods of 8 hours, and the others to dark periods progressively longer by 20-minute increments for each lot, the sixteenth lot receiving dark periods of 13 hours. The remaining eight lots received fifteen dark periods

TABLE 7

RESPONSE OF LARGE AND SMALL XANTHIUM PLANTS SUBJECTED TO VARIOUS DARK PERIOD LENGTHS AT TEMPERATURES OF 40° AND 70°

TEMPERATURE DURING DARK PERIODS (°F.)	DURATION OF DARK PERIODS (HOURS)	LARGE PLANTS		SMALL PLANTS	
		NO. WITH INFLORESCENCES OR FLOWER PRIMORDIA	NO. VEGETATIVE	NO. WITH INFLORESCENCES OR FLOWER PRIMORDIA	NO. VEGETATIVE
40°	8	0	5	0	5
	8⅓	1	4	0	5
	8⅔	0	5	0	5
	9	0	5	0	5
	9⅓	0	5	0	5
	9⅔	1	4	0	5
	10	0	5	0	5
	10⅓	1	4	0	5
	10⅔	1	4	0	5
	11	2	3	1	4
	11⅓	5	0	2	3
	11⅔	5	0	5	0
	12	5	0	4	1
	12⅓	5	0	5	0
	12⅔	4	1	5	0
	13	5	0	5	0
70°	8	0	5	0	5
	8⅓	3	2	0	5
	8⅔	5	0	5	0
	9	5	0	5	0
	9⅓	5	0	5	0
	9⅔	5	0	5	0
	10	5	0	5	0

at approximately 70° F. The first of these lots was also subjected to 8-hour dark periods, and the others to dark periods progressively longer by 20-minute increments for each lot, the eighth lot being exposed to dark periods of 10 hours and 20 minutes.

The results of this experiment (table 7) indicate that the critical dark period at 40° F. was about 11 hours for the large plants and at least 20 minutes longer for the small plants. Since the critical dark

period of the controls (at approximately 70° F.) proved to be about 8 hours and 20 minutes for the large and 8 hours and 40 minutes for the small plants, the lower temperature increased the critical dark period about 2 hours and 40 minutes.

These results indicate, as has other work (1), that as plants grow older they become more sensitive to photoperiodic treatment. Thus at both temperatures the old plants had a shorter critical dark period. Low dark period temperature increases the critical dark period, whether the plants are young or old, by nearly 3 hours. Thus low dark period temperature seems to produce two effects; not only must the plant receive more long dark periods at a low temperature in order to be induced, but each dark period must be relatively longer than is necessary at 70° F.

Effect of saturated atmosphere on critical dark period of Xanthium

Of the 180 vegetative plants selected from the long photoperiod for this experiment, 130 were divided into thirteen lots of ten plants each. For the duration of the experimental period of 10 days, these were maintained during photoperiods in large glass chambers in the greenhouse, where supplementary illumination was provided. Atomizers in the chambers maintained a constant fine spray of water. In order to expose the plants to a dark period of any desired duration in a saturated atmosphere, the various lots were removed from the glass chamber at timed intervals and placed in a darkroom in which the atmosphere was kept saturated. The door to the room was provided with an adequate light and air trap. At 7:30 P.M. one lot of ten plants was removed from the glass chamber and placed in the darkroom. At 7:50 P.M. another lot was placed in the darkroom; at 8:10, another, and so on until 11:30 P.M. The whole group of 130 plants was returned to the glass chamber at 7:30 A.M. Thus the various lots received dark periods varying by 20-minute increments from 8 to 12 hours. All transfers were made as rapidly as possible.

Several determinations of the relative humidity in the darkroom were made with a psychrometer, and no wet bulb depression was observed. As far as is known, all leaves were dripping wet throughout the experimental period, except for two occasions when brief

stoppages in the atomizers in one of the glass chambers permitted the humidity to fall for a short time during two photoperiods.

The remaining fifty plants served as controls. They were divided into five lots which were left on the long photoperiod bench in the greenhouse during the photoperiods of the experimental period, and subjected to ten dark periods in an ordinary darkroom at night.

TABLE 8

RESPONSE OF XANTHIUM PLANTS IN SATURATED ATMOSPHERE AND AT NORMAL GREENHOUSE HUMIDITIES TO VARIOUS DARK PERIODS

ATMOSPHERIC CONDITION	LENGTH OF DARK PERIOD (HOURS)	NO. WITH INFLORESCENCES OR FLOWER PRIMORDIA	NO. VEGETATIVE
Saturated or nearly so	8	0	10
	8 1/3	0	10
	8 2/3	1	9
	9	8	2
	9 1/3	9	1
	9 2/3	10	0
	10	10	0
	10 1/3	10	0
	10 2/3	10	0
	11	10	0
	11 1/3	10	0
	11 2/3	10	0
	12	10	0
Normal greenhouse humidities	8	0	10
	8 1/3	0	10
	8 2/3	10	0
	9	10	0
	9 1/3	10	0

Dark periods were of 8 hours' duration for the first lot, and progressively longer by 20-minute increments for each of the others.

The results (table 8) indicate that very high humidities have only a minor effect on critical day length of *Xanthium*. Other experiments with low humidities (10–18 per cent) indicated similar results.

EFFECT OF LOW LIGHT INTENSITY ON CRITICAL DARK PERIOD OF XANTHIUM

Because of the somewhat irregular responses noticed at times with plants growing in the greenhouse in midwinter, it seemed possible

that low light intensity might affect critical dark period in *Xanthium*. To investigate this possibility, 180 plants were selected from those on long photoperiod. Fifty were left under full illumination as controls, and 130 were placed under a framework covered with several layers of cheesecloth, two layers of which had been tinted a light silver gray color. Light intensity was measured with a Weston light

TABLE 9

FLOWERING RESPONSE OF XANTHIUM PLANTS SUBJECTED TO PHOTOPERIODS AT HIGH OR AT LOW LIGHT INTENSITIES TO DARK PERIODS OF VARIOUS LENGTHS

APPROXIMATE LIGHT INTENSITY IN FOOT CANDLES DURING PHOTOPERIODS OF EXPERIMENT	DARK PERIOD DURING EXPERIMENT (HOURS)	NO. WITH INFLORESCENCES OR FLOWER PRIMORDIA	NO. VEGETATIVE
550	8	0	10
	8⅓	0	10
	8⅔	2	8
	9	6	4
	9⅓	10	0
	9⅔	10	0
	10	10	0
	10⅓	10	0
	10⅔	10	0
	11	10	0
	11⅓	10	0
	11⅔	10	0
	12	10	0
5500	8	0	10
	8⅓	9	1
	8⅔	10	0
	9	10	0
	9⅓	10	0

meter, and at 11:00 A.M. on a clear day was found to vary at leaf surface from 500–600 foot candles on the shaded plants to 5000–6000 foot candles on the control plants. Mazda lamps were included under the cheesecloth so that the supplementary illumination was of the same duration and approximately the same intensity for experimental and control plants.

After 10 days of shading, when the plants were becoming slender and spindling, the critical dark period was determined for both groups. They were divided into lots of ten plants each, and the ex-

perimental plants given ten dark periods which varied in duration by 20-minute increments from 8 up to 12 hours. Controls were also divided into lots of ten plants each and also received ten dark periods in the same darkroom, which was maintained at approximately 70° F. The dark periods for the various lots of controls varied in duration by 20-minute increments from 8 up to $9\frac{1}{3}$ hours. After the treatment all lots, both experimental and controls, were again subdivided, half of each lot placed under the cheesecloth to develop in the shade and the other half allowed to develop under full illumination. All were dissected 2 weeks after the end of the experimental period. Although flowers developed more rapidly in those plants subjected to full illumination after photoperiodic treatment, light intensity subsequent to photoperiodic treatment did not affect the critical dark period, so the last subdivision is not indicated in table 9, where the results of this experiment are reported.

As shown in table 9, the critical dark period of the shaded plants is between $8\frac{1}{3}$ and $9\frac{1}{3}$ hours, but is difficult to determine precisely, since with dark periods of $8\frac{2}{3}$ and 9 hours some of the plants flowered and others did not. The plants grown in full sunlight had a critical dark period between 8 and $8\frac{1}{3}$ hours. Thus plants grown in shade have a somewhat longer critical dark period than plants grown in full sunlight.

Summary

1. The leaves of *Xanthium* plants are the locus of photoperiodic induction. Induced leaves, when grafted to vegetative plants, continue to supply a stimulus for flower initiation when exposed to continuous long photoperiod.

2. If during each 24-hour period, *Xanthium* plants are exposed to 15-hour dark periods at 40° F. alternating with 9-hour photoperiods at approximately 70°, then six to eight such consecutive cycles of dark period and photoperiod are required for photoperiodic induction. A treatment with four such cycles produces a residual effect which lasts more than 24 hours.

3. In these experiments, Biloxi soybeans flowered only after exposure to consecutive long dark periods, each alternating with a short photoperiod. Plants did not flower, regardless of the number of long

dark periods they received, unless at least three of the dark periods were in consecutive order.

4. Soybeans exposed to an induction period of three or more consecutive long dark periods, and which produced as a result a certain number of flower primordia, would not produce new primordia subsequently unless they were again exposed to another induction period of three or more long dark periods.

5. The length of the critical dark period for *Xanthium* is not constant. It decreases slightly in length with increasing age of the plant and varies slightly with variations in humidity. If plants are shaded during their photoperiods their critical dark periods increase slightly in length.

6. Variations in temperature greatly affect the length of the critical dark period, that of plants at 70° F. being approximately $8\frac{1}{3}$ hours while that of plants at 40° is approximately 11 hours.

This investigation was carried on under the direction of and in association with Dr. KARL C. HAMNER, Department of Botany, University of Chicago.

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STRUCTURE OF THE SHOOT APEX IN ZAMIA[1]

MARION A. JOHNSON

(WITH ELEVEN FIGURES)

Introduction

In a recent paper on the shoot apex of *Ginkgo biloba* L., FOSTER (4) has described a structural organization unique for meristems among vascular plants. The essential feature is a centrally placed group of enlarged cells whose origin is traced to several superficial initials occupying the apex of the shoot. These central cells, characterized by their large size, irregular arrangement, large nuclei, and much vacuolated cytoplasm, give rise to the remainder of the shoot apex, which radiates from them as a flanking and rib meristem. FOSTER's suggestion that it would be of interest to explore the situation in the cycads was immediately acted upon by the writer, who had material of *Zamia integrifolia* Ait. and *Z. umbrosa* Small in process of preparation for a study of the origin and development of the leaf tissues. It was soon apparent that a close similarity existed between the fundamental architecture of the shoot meristem in *Zamia* and in *Ginkgo*.

CHAMBERLAIN'S (1) recent volume on gymnosperms reveals that little attention has been devoted to the structure and development of the shoot meristem in cycads. HOFMEISTER (6) in 1857 reported a three-sided apical cell for *Zamia longifolia* (now regarded as a synonym for *Encephalartos longifolius* Lehm.). His figure, however, taken from a transverse section through the apex, is not convincing. Later (7) he grouped the cycads, in a comparison based on the shoot meristems, with those plants having either dolabrate or inverted pyramidal apical cells. STRASBURGER (13) questioned HOFMEISTER's observations and stated that he was unable to discover an apical cell in *Cycas revoluta*. He compared the meristem of *Cycas* with that of the conifers in general, noting in particular the poor de-

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marcation between dermatogen and periblem. He concluded that the relationship between the cryptogamic (with apical cells) and the coniferous type was not to be sought in the cycads but rather in *Lycopodium*. The need for an examination into the meristem of the cycads seemed evident, and it was with this in mind that the present investigation was undertaken.

Material and methods

The data recorded in this paper were obtained from four lots of material collected and fixed as follows. Lot I consisted of about two dozen stem tips of *Zamia integrifolia* and *Z. silvicola* Small, obtained in the vicinity of Floral City, Florida, January 22, 1938, by my colleague Dr. M. A. CHRYSLER. Fixation was in formalin-acetic alcohol. Lot II contained two apices from plants growing in the Rutgers University greenhouse, fixed January 13, 1939, in Navashin's solution. Lot III was collected by the writer in Florida on March 26 to 28, 1939, and included: (1) *Z. umbrosa* from Daytona; (2) *Z. integrifolia* from the region around Fort Lauderdale, in all stages of leaf development, also plants with mature microsporangiate cones and others with megasporangiate cones containing female gametophytes with mature archegonia; (3) *Z. silvicola* from the Hattie Bauer hammock in the vicinity of Miami. The apices were fixed either in the field or during the evening following collection, after having been carefully dissected from the inclosing leaf bases and trimmed to a base 5 mm. square. Satisfactory results were obtained from the use of Navashin's solution, formalin-acetic alcohol and P.F.A.$_3$ (Allen's modification of Bouin's fluid). Lot IV was made up of seeds of *Z. integrifolia* in several stages of germination; fixation was in Navashin's solution. All material was imbedded in paraffin by the tertiary butyl alcohol method as outlined by JOHANSEN (**8**). This treatment facilitated the sectioning of tissues heavily stored with starch.

The drawings have been made with the aid of a Zeiss drawing apparatus, and with the exception of figures 7, 10, and 11 have been reduced from an original magnification of ×430.

Heidenhain's iron-alum haematoxylin, safranin fast-green, and the tannic acid and iron chloride method as recommended by FOSTER (**3**) were satisfactory for staining.

Investigation

Organization of meristem

The short tuberous caudex of the three species studied is terminated by a massive, gently rounded cone or mound of meristematic tissue which is adequately protected by encircling, spirally arranged leaves and scale leaves in varying stages of development. The size and inclination of the sides of the cone are correlated with the stage of development at which the plants are examined. In mature embryos the diameter of the tip 50 μ from the apex measures about 200 μ, while in well grown cone-bearing specimens diameters of 860 and 625 μ at 156 and 78 μ respectively from the apex have been observed. These large meristems have gently sloping sides, are moundlike rather than conelike in shape, and may attain a depth of 460 μ from apex to base of the central zone. The dimensions most commonly observed were a diameter of about 400 μ at 78 μ from the apex and a depth of 300 μ.

The cellular arrangement within the massive apical meristem constitutes four distinct zones, each of which plays a definite role in the development of the mature stem. The relationship between these regions is shown diagrammatically in figure 1. Comparison of this figure with that given by Foster (**4**) for *Ginkgo biloba* shows that the same fundamental plan occurs in both *Zamia* and *Ginkgo*. A clearer understanding of the organization of the meristem as a whole can perhaps best be gained through a brief description of each zone, before a more detailed treatment is attempted.

The apex of the shoot is occupied by a superficial group of cells, the apical initials (zone I), which owing to the number and method of division are not to be confused with the familiar apical cells of the cryptogams. These initials divide by periclinal and anticlinal walls and eventually give rise to all the cells of the meristem. Their immediate progeny, chiefly from periclinal division, accumulate in the center of the meristem as a conspicuous central core (zone II). From the standpoint of origin, position, and relationship to the remainder of the apex, zone II is a distinct unit, but when considered on the basis of function it must be separated into two regions—II*a* and II*b*. Longitudinal sections show the cells in the upper portion (II*a*) to be

relatively small, with dense cytoplasm and heavily stained nuclei; in contrast, those in II*b* are generally larger, with highly vacuolated cytoplasm and faintly stained nuclei. It is evident that in II*a* the principal activity is cell propagation while in II*b* it is cell enlargement.

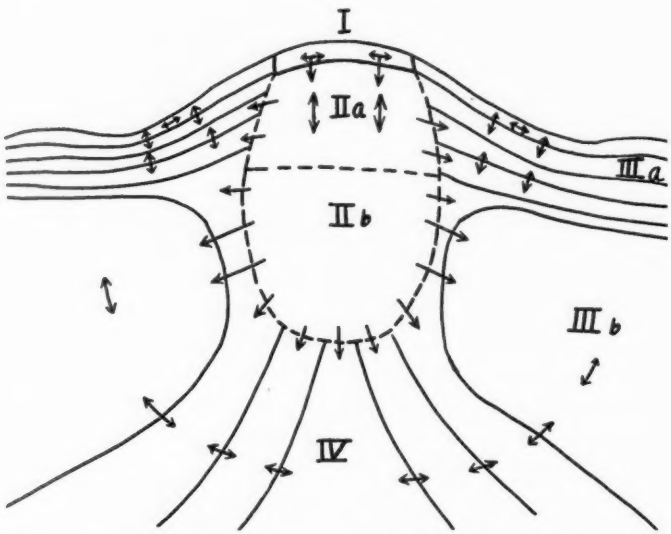


FIG. 1.*—Diagram showing zonal nature of shoot apex of *Zamia* in median longitudinal section. Zone I indicates position of apical initial group. Zone II, which originates from apical initials, represents central mother cells whose upper members (II*a*) are actively dividing while those below (II*b*) are generally in some phase of enlargement. Zone III constitutes the flanking peripheral layers. Zone IV is a rib meristem from which the pith is differentiated. Note importance of central mother cells (zone II) in contributing to remainder of meristem. Arrows indicate general direction of growth in various zones.

* Legends for drawings: *a*, apical initials; *p*, periclinal divisions in surface layers; *pl*, peripheral layers (zone III); IIa, actively dividing central mother cells; IIb, enlarging central mother cells; *ppl*, periclinal divisions in cells derived from surface layer; *r*, rib meristem; "wall thickenings" in black.

It should be emphasized that zone II is the most characteristic feature of the entire meristem. FOSTER (4) has accordingly proposed that it be known as the central mother cell zone in *Ginkgo*. His term is equally appropriate for *Zamia*, since here also both zones III and IV show by their radial cellular alignment that their origin for the

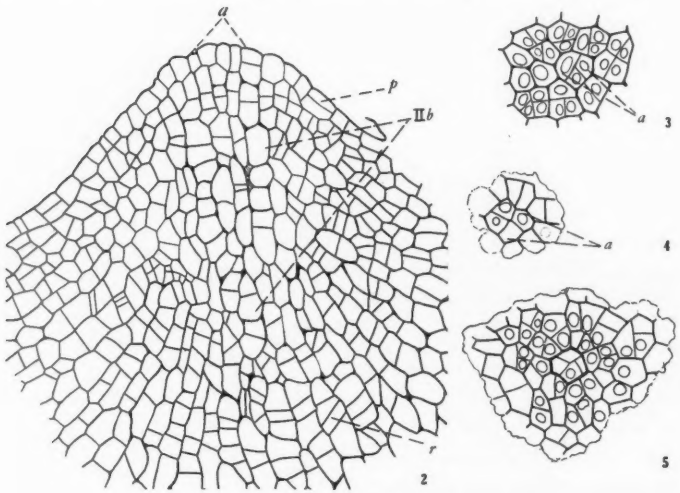
most part can be properly traced to this central region. Zone III is a flanking meristem surrounding the central mother cells, and ultimately matures into epidermis, cortex, vascular tissue, and probably the outer portion of the pith. Zone IV, on the other hand, consists of a shallow rib meristem underlying the central mother cells. It soon differentiates into the remainder of the conspicuous pith.

Zone I: Apical initials

Determination of the exact location of an apical cell group must of necessity depend upon a study of median longitudinal and transverse sections. Considerable difficulty was met in this respect. In *Zamia* the apex is low and broad; in figures 2, 6, and 8 diameters of 150, 175, and 250 μ respectively are found 30 μ from the apex. Therefore, longitudinal series cut 8 μ in thickness show five or six sections having a similar structure, any one of which might be identified as median. Great care was exercised to make certain that each section used for drawings was truly median. Figures 2, 6, and 8 show that the apex of each shoot is covered with a single layer of cells sometimes somewhat larger than those flanking them. Their number is highly variable; for example, in conical apices (figs. 2, 6) there are probably six to eight respectively in each median section. If these tips are symmetrical in shape, as is usually the case, the entire number would be fifty or more. Broad mound-shaped tips similar to that in figure 8 (and which may be much larger in old specimens of *Z. integrifolia*) must approach one hundred. On the other hand, in an embryo (fig. 7) the number probably will be under a dozen. The exact center of the tip, if it were possible to locate it with certainty, would probably be occupied by a cell capable of forming a tetrad similar to that shown in figure 4. Figure 5, the upper surface of which is just 8 μ below figure 4, indicates that the base of the superficial tetrad is in the center of the figure and surrounded by some forty cells dividing in a similar manner. These are the initial cells as viewed in transverse section, and owing to their method of cleavage, form an irregular, cellular pattern clothing the summit of the shoot. While these cells are more or less identical in size, content, and method of cleavage at a given time, it is only the ones at the exact apex that continue to supply cells throughout the life of the

plant and which are thus the true apical initials. Those on the periphery are gradually crowded to the outside and eventually become engulfed in the production of leaf primordia.

In view of the fact that HOFMEISTER (7) considered the cycads to have either dolabrate or pyramidal apical cells, it might be wise to examine the method of segmentation in the apical initial group. It



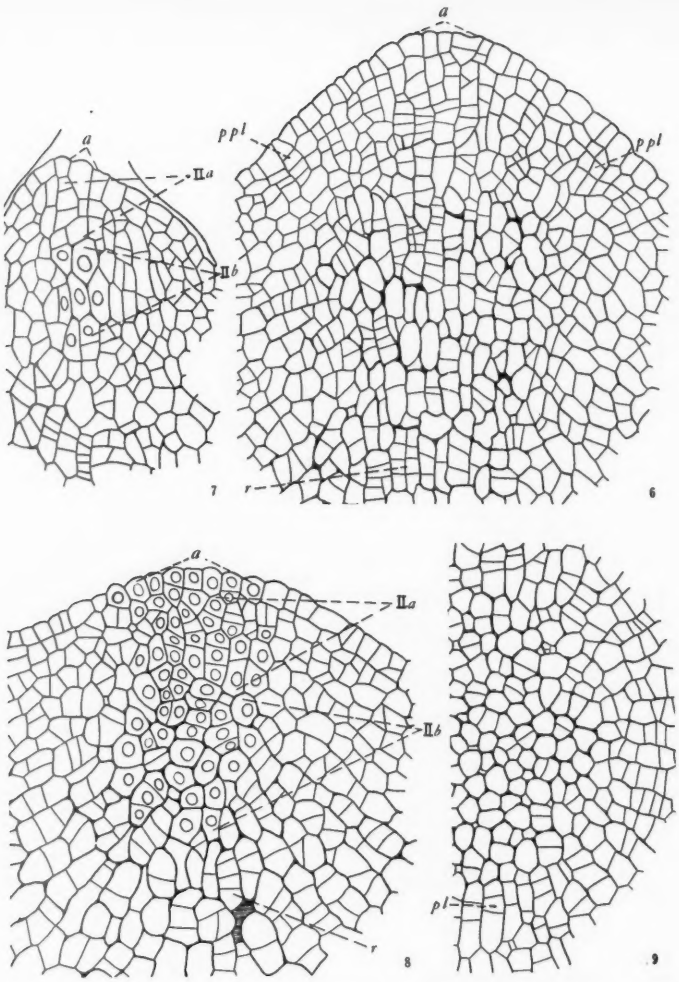
FIGS. 2–5.—Fig. 2, *Z. silvicola*. Median longisection showing cone-shaped meristem. Note vertical alignment in central mother cell zone resulting from periclinal divisions; practically all cells in remainder of meristem radiate from central mother cells. Fig. 3, *Z. umbrosa*. Transection showing cells of apical initial group. Three cells at *a* interpreted as being near geometric center of apex. Note tendency for initials to form tetrads by anticlinal division. Fig. 4, *Z. integrifolia*. Transverse section through cone-shaped apex. Note tetrad of apical initials. Fig. 5, section taken 8 μ below fig. 4. Note base of apical tetrad in center and irregular arrangement of surrounding initials.

will be recalled that a dolabrate cell cuts off segments alternately by anticlinal division from each of its curved lateral faces, and that in the pyramidal type anticlinal walls carve segments from each of the three lateral triangular faces in sequence. Figures 3 and 4, transverse sections through the apex, show that in *Zamia* the initials may be divided into tetrads by anticlinal walls. Furthermore, when viewed in longitudinal section (fig. 8) it is seen that periclinal divisions occur,

which is not the case in either dolabrate or pyramidal apical cells. It follows, therefore, that the structural affinities of the apical initial complex lie not with the cryptogams as a whole but rather with the scheme shown by FOSTER (**4**) for *Ginkgo;* by KORODY (**10**) for *Abies*, *Picea*, and *Pinus;* and by HÄRTEL (**5**) for a number of species of *Lycopodium.*

ZONE II: CENTRAL MOTHER CELLS

This zone is of special interest because, with the exception of *Ginkgo*, it appears to be unique among plants. The general scheme of the cell pattern, as it appears in median longitudinal section, may be obtained from figures 2, 6, 7, and 8. The first impression is that of a central core of vertically aligned cells extending some 200 to 400 μ into the heart of the meristem, from which the cells of zones III and IV radiate in more or less regular rows. The apical initials contribute to the central mother cells by periclinal divisions. The inner layer of daughter cells behaves much as initials, in that the cells divide both anticlinally and periclinally (the latter divisions predominate), so that tiers of cells from four to twelve layers deep are built up. This region, designated in figure 1 as zone II*a*, is apparently one of cell multiplication. Unfortunately direct evidence for the actual rate of division has not been found in any of the material examined. The only mitotic figures observed were in two seedlings from lot IV. In view of the number of apices studied and the variety of fixing agents used, some explanation is necessary to account for the failure in locating mitosis. The plants of *Zamia integrifolia*, which were fixed immediately in the field, bore leaves and cones in many stages of development. Care was used in removing the tissues surrounding the bases of the tips in order to insure rapid fixation. Material from the greenhouse, and a few plants which had been brought into the laboratory one week after collection in the field, were fixed under reduced air pressure to make quick penetration certain. Negative results were obtained in both cases. The interval between collection and fixation was apparently not excessive, for COULTER and CHAMBERLAIN (**2**) obtained mitosis in ovules from cones which had been in the laboratory for two weeks. Penetration must have been satisfactory, for division figures occurred in seed-



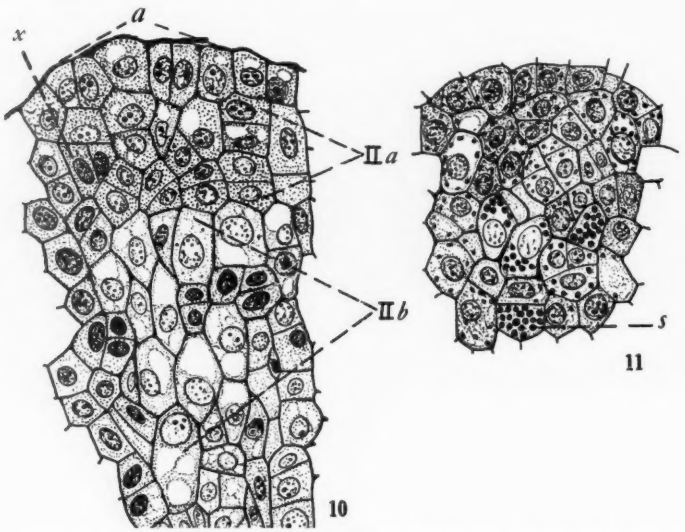
FIGS. 6–9.—Fig. 6, *Z. umbrosa*. Median longisection with gently rounded apex. Note tiers of cells in peripheral layers derived from superficial layer (*ppl*), also extensive development of central mother cell zone with oblique-anticlinal cleavage contributing to zone III. Fig. 7, *Z. integrifolia*. Median longisection through shoot apex of mature embryo. Note enlarged central mother cells, periclinal divisions in superficial layer, and large apical initial which might be mistaken for an apical cell. Drawing made from slide prepared by Dr. M. A. CHRYSLER. Fig. 8, *Z. integrifolia*. Median longisection through mound-shaped apex. Note strong tendency for cells to radiate from central mother cell zone and oblique anticlinal divisions through which they have been derived; also origin of rib meristem from rejuvenated central mother cells; and that cells derived from superficial layer may divide anticlinally. Fig. 9, *Z. integrifolia*. Transection through inner peripheral layers showing radial arrangement and origin from central mother cells.

lings fixed with the same treatment as that used for the mature plants. The discovery that the majority of the apices were gorged with starch suggested that protein synthesis had been retarded sufficiently to throw the meristematic tissues into a period of dormancy so far as mitosis is concerned. This seems likely when it is recalled that the cycads are noted for their exceedingly slow growth and their habit of suddenly producing cones or a crown of leaves following periods during which signs of growth were not visible. Material would have to be collected over a longer period of time than has been available in order to determine the duration of the dormant and active periods. Thin cell walls, large, deeply stained nuclei and dense cytoplasm, in addition to paired cells, have been considered as sufficient proof for indicating recent division.

The actively dividing cells (zone II*a*) by either gradual or abrupt transition merge into zone II*b*, which contains the most conspicuous cells in the meristem. In median longitudinal sections they are seen to be about twice as long as their peripheral neighbors, and to have thin, highly vacuolated cytoplasm and large, faintly stained nuclei (fig. 10). Deeply stained thickenings often occur where several cells make contact. This feature is not nearly so prominent as has been figured by FOSTER (4) for *Ginkgo*, and may be absent (fig. 10). The enlarged cells are seen less readily in transverse section, owing in part to the fact that all the cells have about the same diameter. Critical examination of cell contents, however, reveals their presence (fig. 11). Division may occur deep within the central mother cell zone, cleavage being anticlinal, periclinal, or oblique (figs. 10, 11). As a result the enlarged cells become isolated among small ones and the conspicuous feature of the zone seems to have disappeared. Thus while zone II*b* is generally regarded as a region of enlargement, it might be more accurate to think of enlargement as an ultimate characteristic of the central mother cells before they return to the meristematic condition leading to final differentiation. For the most part the enlargement phase is reached by the majority of the cells at the same time, but exceptions occur, as already noted.

The central mother cell zone, when considered as a whole, is obviously a region of increase in volume, accomplished by both cell division and cell enlargement. Its most significant characteristic,

regardless of its structure or stage of cellular activity, is that it serves as the point of origin for most of zone III and all of IV. No exception has been found to this statement in the fifty tips examined. On the other hand, nothing approaching this condition can be seen in the excellent figures of conifer apices in the comprehensive work of KOCH (9) or KORODY (10).



FIGS. 10, 11.—Fig. 10, *Z. integrifolia*. Median longisection showing portion of central mother cells in detail. Note anticlinal divisions in apical initials; periclinal and oblique periclinal divisions which build up actively dividing part of central mother cell zone; anticlinal division in central mother cell at *x* contributing to superficial layers; enlarged central mother cells with large faintly stained nuclei and vaculoated cytoplasm; also small cells from recent division within central mother cell zone. Fig. 11, *Z. silvicola*. Transection through enlarged cells of central mother cell zone. Note prominent starch grains (*s*).

ZONE III: PERIPHERAL LAYERS

The peripheral layers flank the central mother cells and owe their origin to them and the outer layer of the meristem below the apical initials. In apices containing starch this zone can be subdivided into III*a* and III*b*. The lower part (III*b*) is composed of some six rows of cells (fig. 8) radiating from the lower portion of the central mother

cell group. Division is somewhat obliquely periclinal, and the newly formed cells appear to be in filaments revealing their common origin from a single mother cell. New filaments or rows of cells may be started from divisions parallel to the surface which tend to broaden the peripheral layers and give them a fanlike appearance. Transverse sections taken at this level (III*b*) supply convincing evidence that the radiating rows of cells have their origin in a transition zone belonging to the central mother cell group (fig. 9).

The outer peripheral layers, generally about four in number, are derived from two sources. Some of the cells result from anticlinal divisions in the upper four or five layers in the central mother cell zone which have not passed through the enlargement phase characteristic of the members deeper within that zone; the others have been derived from periclinal divisions in the surface cells. The daughter cells from the latter source divide periclinally, and build up tiers of cells three and four layers in depth (figs. 2, 6, 8). *Zamia* differs from *Ginkgo* in that a much greater contribution is made to the peripheral layers by these cells.

Continued development in the peripheral layers results in formation of leaf primordia, cortex, vascular tissue, and probably the outer part of the pith, in the order named.

Zone iv: Rib meristem

Schüepp (12) has introduced the term rib meristem to indicate a primary meristem concerned with growth in length. The cells are arranged in vertical rows which have been produced by continued transverse division of the mother initials. The term is used here even though elongation in the stem of the Florida species of *Zamia* is much reduced.

The central mother cells lie directly above the pith and contribute to it through the medium of a shallow rib meristem (figs. 2, 6, 8). The divisions are periclinal and form short filaments, but vertical cleavage also occurs and thereby increases the diameter of the meristem. The presence of a rib meristem indicates convincingly that the enlarged central mother cells represent a phase in the growth of the meristem proper, for without it they would be regarded as maturing pith cells.

Occurrence of starch

The presence of starch is not unexpected in the tissues of a plant which has the reputation of being one of the few North American species exploited commercially for this product. It is surprising, however, to find that the meristem which is normally concerned with protein synthesis should become gorged with stored carbohydrate. Thin sections through the shoot apices from lot III, when treated with iodine, gave a positive reaction for starch in all four zones, although it was most pronounced in zones III and IV. In some cases the entire superficial layer gave a strong positive test, while in others only a few cells in the central mother cell zone contained starch. In general, zones I, II, and III*a* were most likely to be starch free. This made them stand out and assume an outline not unlike that of a circular topped glass stopper inserted into the broad cone-shaped apex.

The storage of starch throws light on certain problems in meristem function as a whole. First, it shows that a meristem may be shifted from protein to carbohydrate synthesis with a period of dormancy as a result. Second, it shows that large amounts of soluble food must readily penetrate the meristem, which would be of great importance where the meristem is a massive structure as in *Zamia*. If the cell wall serves in this capacity, as has been proposed by Priestley and Tupper-Carey (11), it must offer an efficient pathway for translocation even though the time for penetration may be long. Third, it shows that the conversion of the starch reserves to soluble form provides food for rapid growth in all parts of the meristem, and thus reduces the necessity of translocation over long distances, however it may occur.

Discussion

Fundamentally the architecture of the shoot apex is the same in the Florida species of *Zamia* as in *Ginkgo biloba*. Each has a meristem composed of four distinct zones, the combination of which is not found elsewhere among plants. The apex is more massive in *Zamia* than in *Ginkgo* and it continues to increase in size as the plant ages and the maximum number of leaves per whorl is reached. The largest specimen examined had a cone with partly matured seeds, a crown of fourteen well developed leaves, and an apical meristem

measuring 856 μ in diameter 156 μ from the apex. A comparison zone by zone shows that the apical initials are more numerous in *Zamia;* that the central mother cells are more active near the apex and are more inclined to divide periclinally than in *Ginkgo*, with the result that they accumulate in vertical rows as seen in median longitudinal sections. This subzone becomes a well marked feature in *Zamia*, but is represented in *Ginkgo* by the subapical layer of cells which in part may function as initials by dividing periclinally. In *Zamia* periclinal divisions are more common than anticlinal. The latter do occur, however, especially at the periphery of the region. The lower central mother cells are more inclined to be in vertical rows, and are more often isolated by small cells than in *Ginkgo*. The outer layers of the peripheral meristem have their cells arranged more nearly in vertical tiers than has *Ginkgo*, which suggests that they have been derived largely from periclinal divisions in the surface layers. The rib meristem is shallow and compares favorably with that in the spur shoots of *Ginkgo*.

FOSTER (**4**), comparing the shoot apex of *Ginkgo* with that of other gymnosperms, concludes that the closest similarity lies in the Abietaceae, which have an apical cell group resembling that of *Ginkgo* in form and behavior, and furthermore lack a typical "dermatogen." Comparison between *Zamia* and the Abietaceae warrants the same conclusion, but it must be borne in mind that the studies on the Abietaceae by KOCH (**9**) and KORODY (**10**) fail to show the presence of a zone comparable in any respect with the central mother cells from which the remainder of the meristem radiates in *Ginkgo* and *Zamia*.

The more nearly vertical arrangement of cells in the central mother cell zone might be argued as placing *Zamia* nearer to the condition attained in the Abietaceae. This similarity is not advanced as illustrating relationship, since the unique characteristic of a central region serving as "cambium" for the remainder of the meristem is not approached in the conifers.

No attempt, even if feasible, can be made at this time to determine the degree of evolutionary relationship between *Ginkgo* and the cycads based on the organization of the shoot apex. STRASBURGER (**13**) figures (table 25, fig. 36) a longitudinal section through the stem tip of *Cycas revoluta* in which the existence of a central zone somewhat

comparable with the central mother cell zone in *Zamia* is suggested but by no means proved.

It would follow, should the condition as seen in *Zamia* be typical, that the occurrence of such similarity of structure in forms as widely separated as the cycads, which were derived from the Cycadofilicales, and *Ginkgo* with its ancestry in the Cordaitales, will doubtless be of significance in an understanding of the evolutionary development in the shoot apex of gymnosperms.[2]

Summary

1. The shoot meristem of *Zamia integrifolia*, *Z. silvicola*, and *Z. umbrosa* consists of four distinct zones, as follows:

Zone I, a group of superficial initials occupying the broad apex of the shoot. The entire meristem has its origin in these cells.

Zone II, a central core of mother cells derived by periclinal divisions from zone I. This is a region of increase in volume accomplished by rapid cell division in its upper layers and by cell enlargement in the lower. These later are rejuvenated and contribute to the surrounding meristematic zones. *Ginkgo* seems to be the only other vascular plant so far investigated which duplicates this region.

Zone III, a series of peripheral layers flanking zone II. The outer layers originate from the superficial cells of the apex and the inner ones by oblique divisions of the central mother cells. Leaves, cortex, vascular tissue, and probably a portion of the pith originate in this zone.

Zone IV, a shallow rib meristem of vertically aligned cells, produced entirely from the base of the central mother cell zone and separating it from the maturing pith.

[2] The recent work of FOSTER (FOSTER, A. S., Structure and growth of the shoot apex of *Cycas revoluta*. Amer. Jour. Bot. **26**:372–385. 1939) has not been discussed since it was not received until after the present paper had been submitted for publication. A close similarity exists in the structure and zonation of the shoot apex in *Cycas* and *Zamia*. My interpretation in *Zamia* has been based on the general plan seen in *Ginkgo* but with sufficient modifications to bring it in line with the interpretation given by FOSTER for *Cycas*. The principal difference seems to be in the growth of the peripheral layers; in *Cycas* the cell pattern is highly variable, as is also the case for young *Zamia* plants. In large specimens with moundlike apices the cell pattern is still variable, however, but there is a well marked tendency for the cells to be stratified in almost vertical rows. These originate from periclinal (to the apex) divisions of the superficial layer as well as of the layers beneath.

2. The occurrence of starch throughout the shoot apices in plants collected during January and March suggests that the meristem undergoes alternating periods of dormancy and active growth.

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HISTOLOGICAL AND PHYSIOLOGICAL RESPONSES OF BEAN PLANTS TO ALPHA NAPHTHALENE ACETAMIDE[1]

E. J. KRAUS AND J. W. MITCHELL[2]

(WITH FIFTEEN FIGURES)

Introduction

During the past several years, thirty-six growth promoting substances have been tested to determine their effect on the histological development of plants to which they were applied, and also to test some of their physiological effects. Some of these compounds have been listed by TRAUB (10) with respect to their effect in promoting root development when applied to stem cuttings. Among those tested, one, alpha naphthalene acetamide, m.p. 184°, produced by the American Chemical Paint Company, resulted in such striking and characteristic effects that it has seemed worthwhile to invite attention to the results of several experiments in which it has been used.

This substance is but sparingly soluble in either water or lanolin. It was employed in the following ways: (1) it was applied to the cut surfaces of bean, African marigold, and *Mirabilis* stems as a 2 per cent mixture in anhydrous lanolin; (2) it was sprayed on to the surfaces of these and other species as emulsions of varying concentration; and (3) cuttings of various plants were partially immersed for varying lengths of time in aqueous solutions of it.

When the 2 per cent lanolin mixture was applied to the cut surfaces of young, 3 cm. long, decapitated second internodes of the bean or to first internodes of *Mirabilis*, the gross responses were strikingly different from those of all other substances tested. Following application there was some further elongation of the internodes, very little proliferation of tissues to form an apical tumor, and a few roots

[1] This investigation was aided in part by a grant to the University of Chicago from the Rockefeller Foundation.

[2] Associate Physiologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U.S. Department of Agriculture.

emerged near the surface of application, especially if the treated plants were subsequently placed in a saturated atmosphere (5). Other noticeable features were the greatly increased secondary thickening of the entire stem below the surface of application, especially the differentiation of secondary xylem, the decided thickening of the walls of the cells of most of the tissues composing the stem, and the partial suppression of elongation of axillary buds.

The most obvious characteristic was the greatly increased firmness or hardness of the treated internodes and in the case of *Mirabilis* the failure of the treated internodes to absciss even after many weeks, although ordinarily they will do so in eight or ten days when treated with lanolin only or if decapitated and left untreated. A delay of abscission has already been noted as one of the effects of application of lanolin indoleacetic acid mixtures to *Mirabilis* (2), but the effect is much less pronounced or lasting than when alpha naphthalene acetamide is used. In the case of the latter, secondary thickening takes place so quickly and so extensively after its application either in lanolin mixture or as a spray, and these effects are so extensively distributed throughout the plant (fig. 1), that its use may well prove of economic importance when it is desired to increase the degree or rate of secondary thickening or in the prevention or delay of abscission of plant organs such as leaves, flowers, fruits, etc. Its effect on parthenocarpy in holly has been demonstrated by GARDNER and MARTH (1).

In the illustrations which are used in this paper, the specific experimental treatment imposed is given in the legend for each figure and the histological details are generally obvious. As already stated, one of the most evident features is the effect of the treatments on the degree and rate of increase of the xylem and on wall thickening. The root systems of treated as compared with untreated plants are much more fibrous. When roots are produced by the stems following application of a 2 per cent lanolin paste, their origin is generally from the derivatives of the cells of the rays (figs. 2, 4). Frequently the endodermal cells proliferate and from them vascular bundles are differentiated (fig. 5). The cells of the pith in the vicinity of the vessels also may divide (fig. 8), and from these vascular strands may be differentiated. The latter effects are similar to those resulting from ap-

plications of indoleacetic acid lanolin mixtures (**3, 5**) but are much less pronounced within the same period of time after treatment. Because of its relatively low solubility, the effective concentration of alpha naphthalene acetamide which may penetrate to or reach cells at some distance from the point of application may also be low. But it is clearly obvious that even though the concentration is low there is a ready penetration of the tissues for appreciable distances from the surface of application. This is indicated in the responses shown by entire plants whose leaves have been sprayed with emulsions of it (fig. 1) and in the increased activity of the cambium, the amount of secondary xylem differentiated, and the extensive thickening of the cell walls (fig. 7).

In addition to the numerous experiments in which applications were made to stems and other parts of various species of plants to study the subsequent histological development, several physiological experiments were conducted to determine: (1) the effect of alpha naphthalene acetamide on bud inhibition; (2) its effect on the accumulation of solid substances in the part of the plant to which the chemical was applied; and (3) the histological responses as compared with those resulting from indoleacetic acid. Since the results of comparable experiments have been similar, details of only two are presented here.

Investigation

Experiment I

Kidney bean plants were grown in soil in the greenhouse until the second internodes were 2.5–5 cm. long and the first trifoliate leaf was beginning to expand and flatten out. All the plants were then decapitated by severing the second internodes about 1.5 cm. above the node at which the heart-shaped leaves are borne. They were then divided into four equal lots. To the cut surfaces of one lot a thin smear of a 2 per cent mixture of lanolin-alpha naphthalene acetamide was applied, to another a 2 per cent mixture of lanolin-indoleacetic acid, to a third lanolin only, and the fourth was left without further treatment. The several treatments were given at random over two large benches, and involved several hundred plants.

One week later the plants were harvested and divided into the fol-

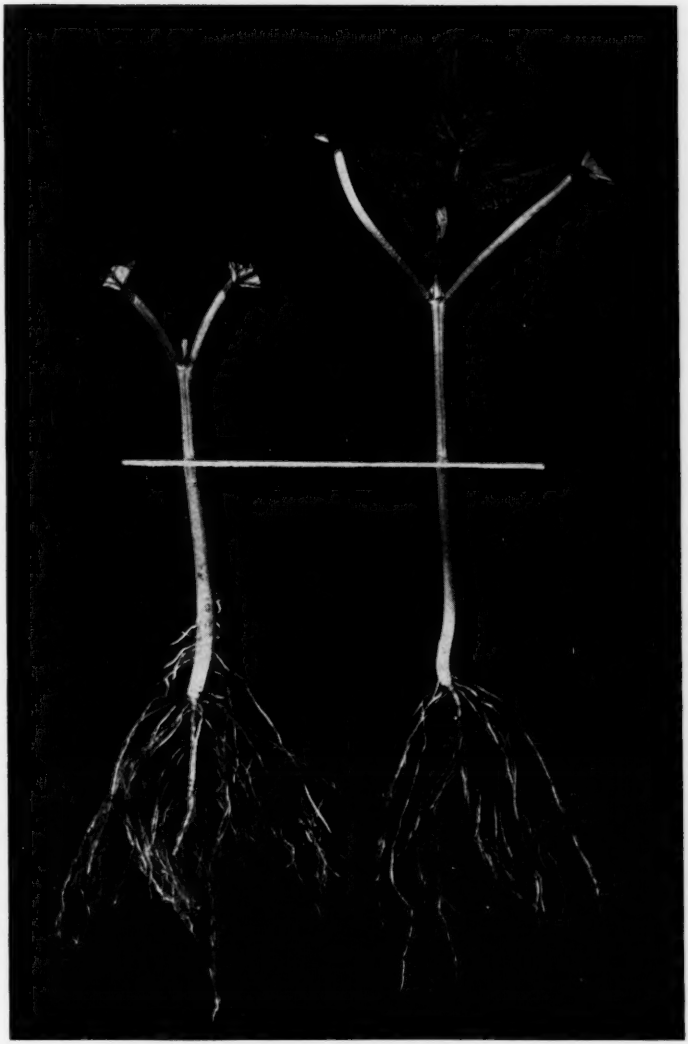

FIG. 1.—Plant (at left) 5 days after primary leaves had been sprayed with emulsion of alpha-naphthalene acetamide, 625 mg. per liter; plant at right sprayed with lanolin emulsion only. Primary leaves 3–4 cm. long; second internode not yet elongated at time of spraying. Blades of primary leaves removed when photographed. The white line passes through the cotyledonary node. The lesser elongation and increased thickness of all above-ground parts (except second internode); the finer, more fibrous root system; and the initiation of roots on the stem below the primary leaves are characteristic effects following spraying with this growth substance.

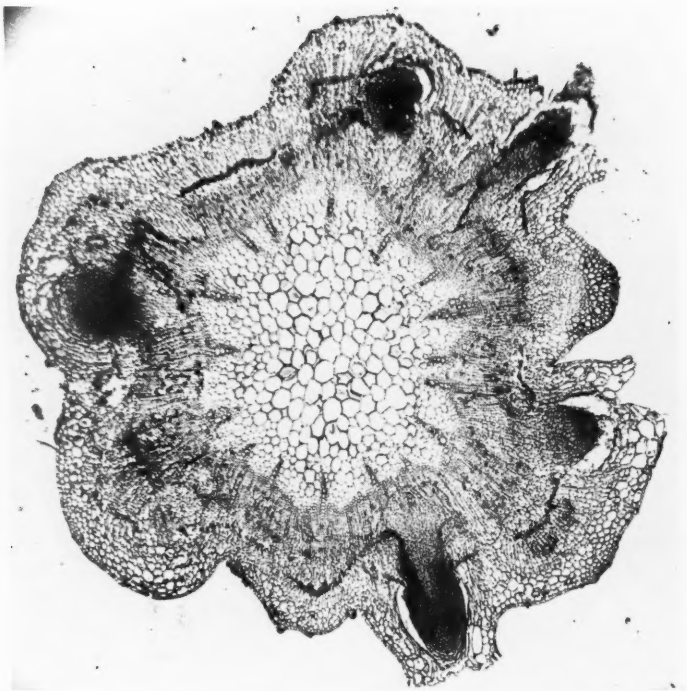

FIG. 2.—Transverse section (about 1.5 mm. below treated surface) of second internode of decapitated bean plant 20 days after treatment of cut surface with 2 per cent alpha naphthalene acetamide-lanolin mixture. Plants maintained for last 5 days of period in humid atmosphere. Relation of roots to rays, a few vascular strands from endodermal derivatives, and great increase in secondary xylem are characteristic. *Cf.* fig. 3.

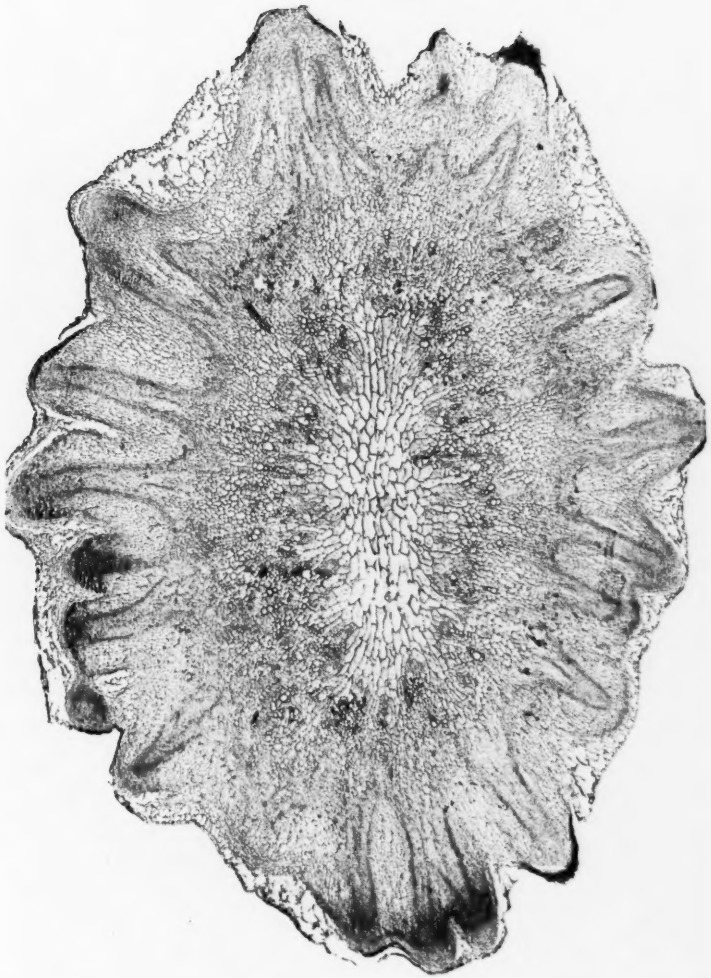

FIG. 3.—Transverse section (about 1.5 mm. below treated surface) of second internode of bean plant 7 days after treating cut surface with 3 per cent indoleacetic acid-lanolin mixture. Compared with section shown in fig. 2, the much greater development of roots and the greater proliferation of pith, phloem, and other tissues are characteristic.

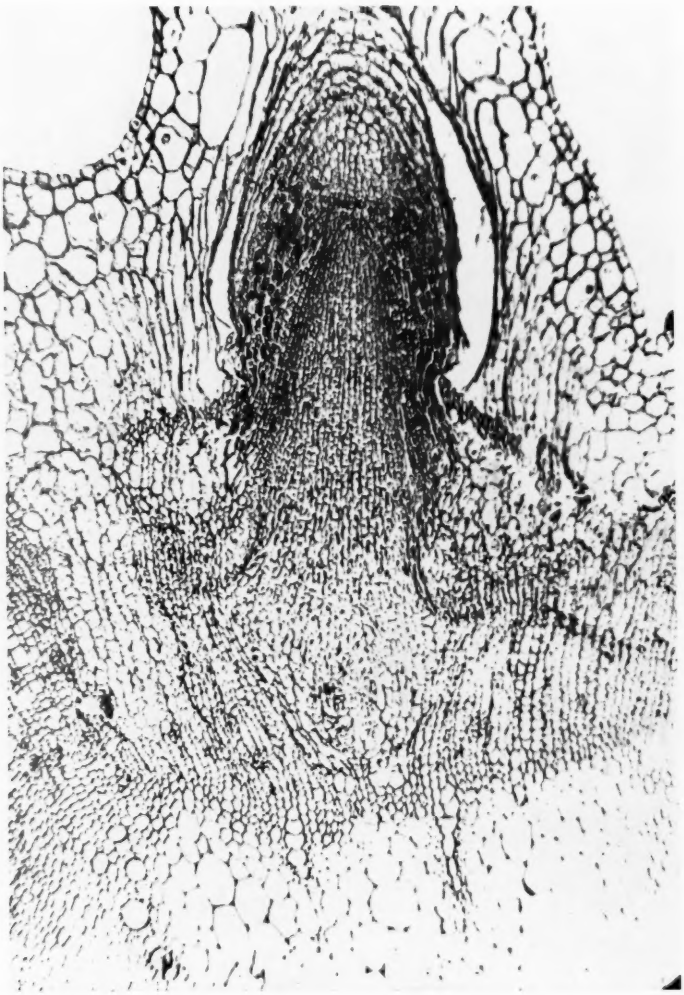

FIG. 4.—Enlarged view of young root shown in fig. 2. Pericyclic fibers are thick walled and disrupted by the root having passed through them.

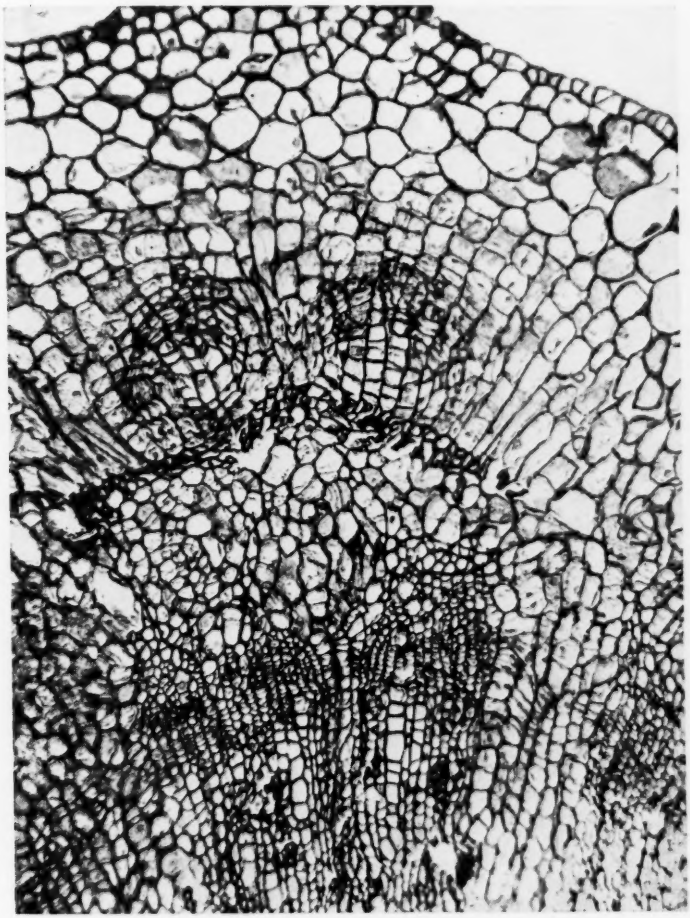

FIG. 5.—Portion of section shown in fig. 2 greatly enlarged. Two vascular bundles differentiating from derivatives of endodermis. Pericyclic fibers much thickened; primary phloem only slightly meristematic.

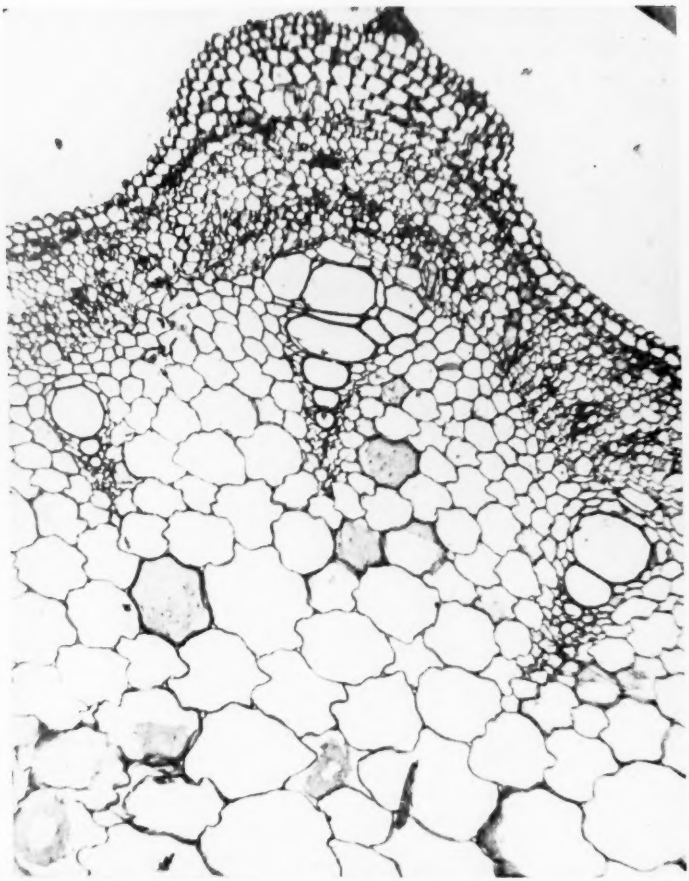

FIG. 6.—Section of second internode decapitated when trifoliate leaf had just flattened and treated surface smeared with lanolin. Section 15 mm. from treated surface, 20 days after treatment. There has been relatively little increase in secondary xylem, although there has been a partial maturation of most other tissues. *Cf.* fig. 7.

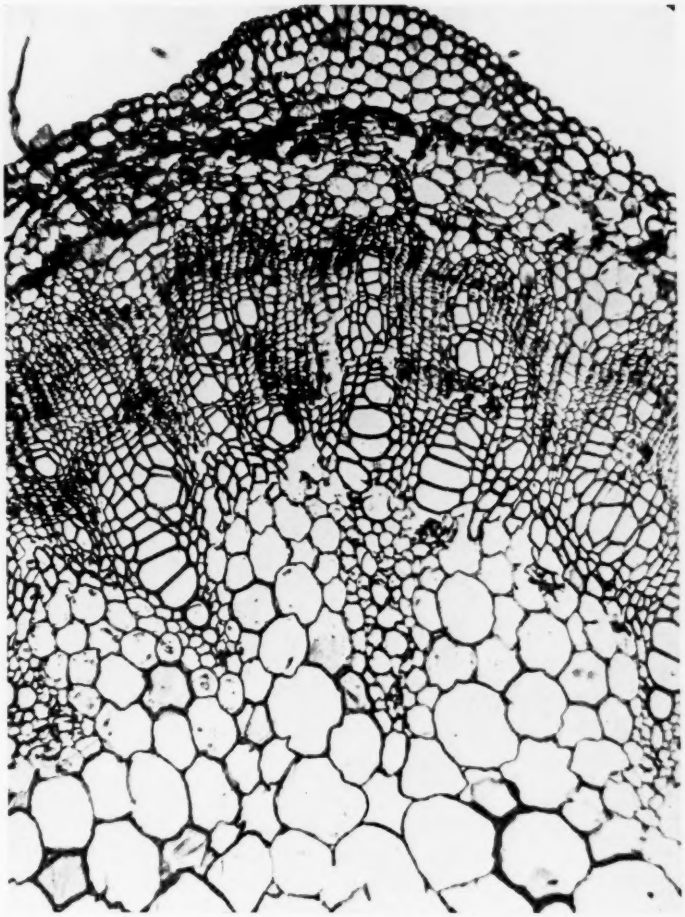

FIG. 7.—Internode of same age as one shown in fig. 6, cut surface treated with 2 per cent alpha naphthalene acetamide-lanolin mixture. Section 15 mm. from treated surface, 20 days after treatment. Relatively great development of secondary xylem and increased thickening of walls of cells of other tissues are characteristic.

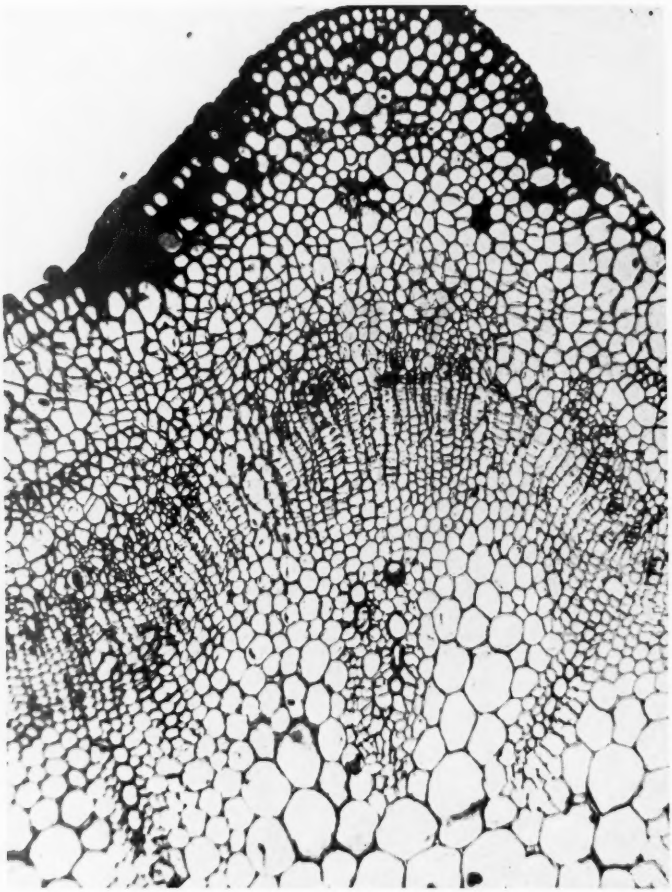

FIG. 8.—Same internode as shown in fig. 7 except that the section is just immediately below the treated surface. There is some proliferation of pith cells adjacent to xylem vessels, of phloem and cortical cells, and some cells have died. Immediately below this level there is increased secondary thickening for long distances down the treated internode and throughout other portions of stem below it. *Cf.* fig. 7.

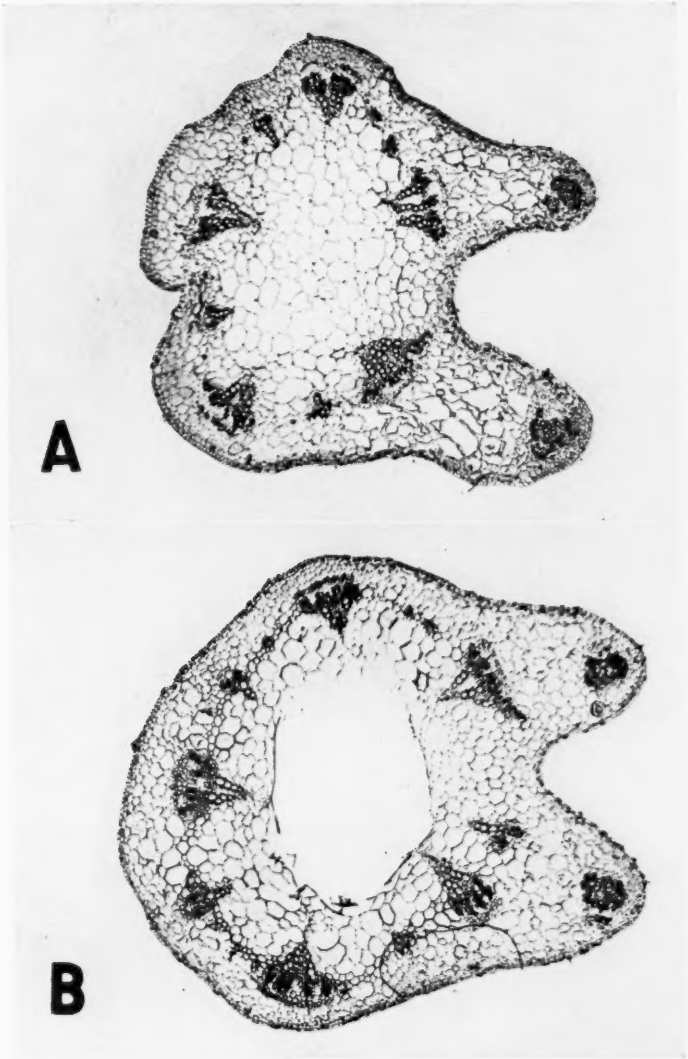

FIG. 9.—Petioles from bean plants used in experiment II. *A*, sprayed with emulsion containing lanolin only; *B*, with alpha naphthalene acetamide-lanolin emulsion; both harvested 8 days after first application of spray. Greater diameter of petiole, greater amount of secondary xylem, increased thickness of cell walls, and tearing of parenchyma at center (as shown in *B*) are characteristic. *Cf.* figs. 10 and 11.

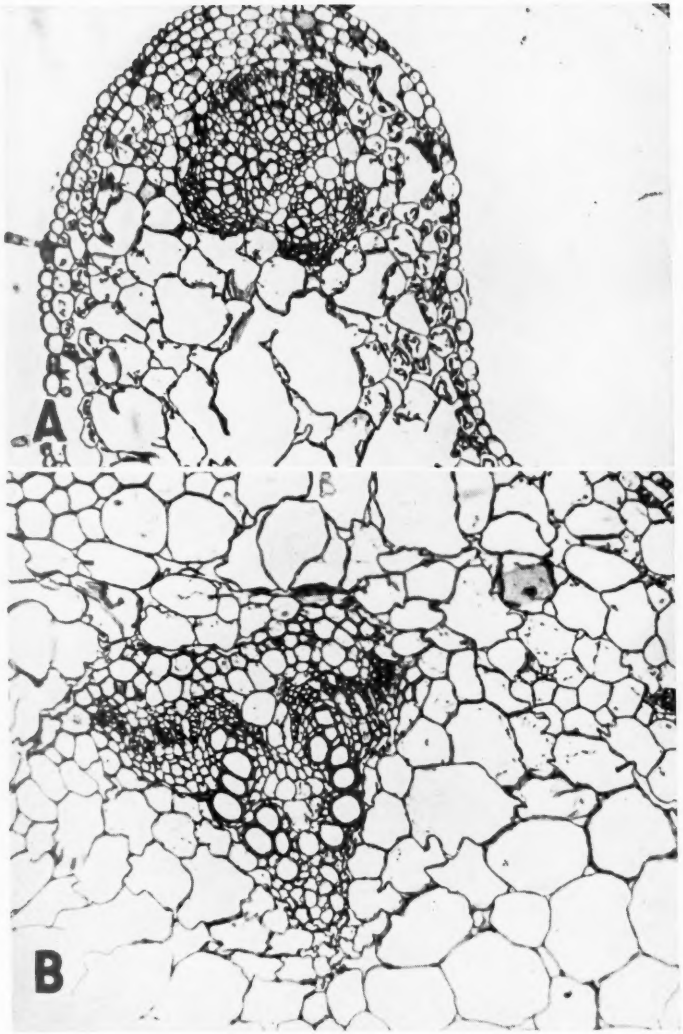

FIG. 10.—Portions of petiole shown in fig. 9*A* greatly enlarged. *A*, vascular bundle in ridge of petiole; *B*, one of larger bundles just above middle at left in fig. 9*A*.

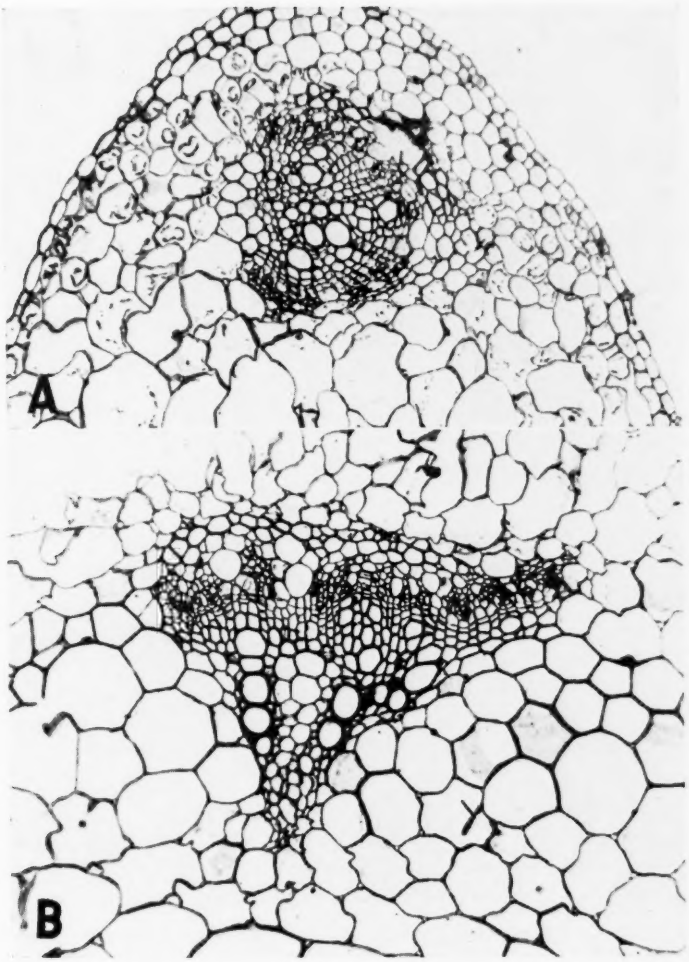

FIG. 11.—Portions of petiole shown in fig. 9*B* greatly enlarged. *A*, *B*, bundles in same relative position as described in fig. 10. Greater thickness of walls of parenchymatous cells and increased secondary xylem are characteristic.

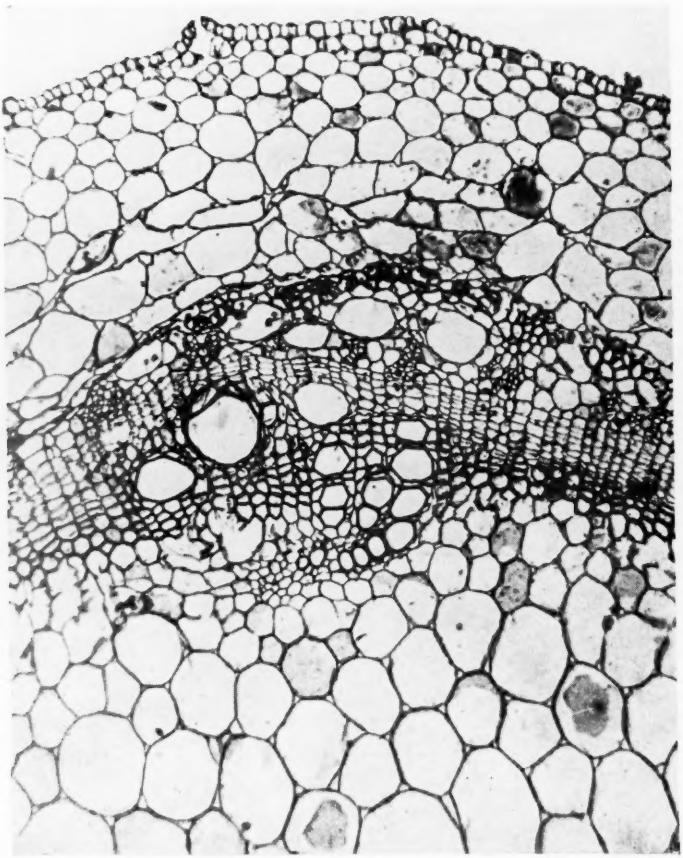

FIG. 12.—Transverse section of mid-region of hypocotyl of plant of experiment II 8 days after first spraying primary leaves with lanolin emulsion.

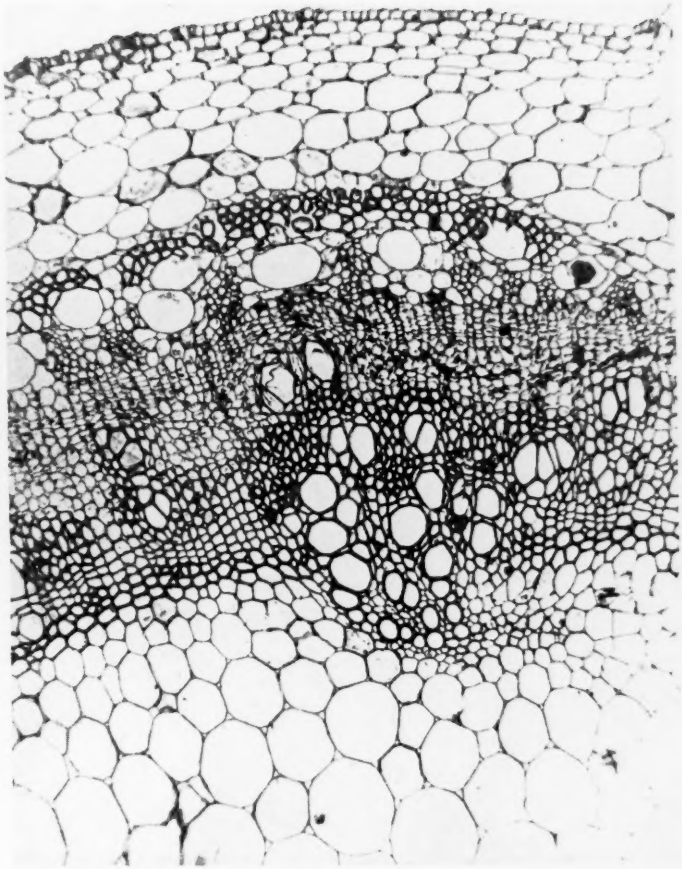

FIG. 13.—Transverse section of mid-region of hypocotyl of plant of experiment II 8 days after first spraying primary leaves with emulsion of lanolin-alpha naphthalene acetamide. *Cf.* hypocotyl shown in fig. 12; amount of secondary xylem is very much greater as are also the wall thickenings of several other tissues.

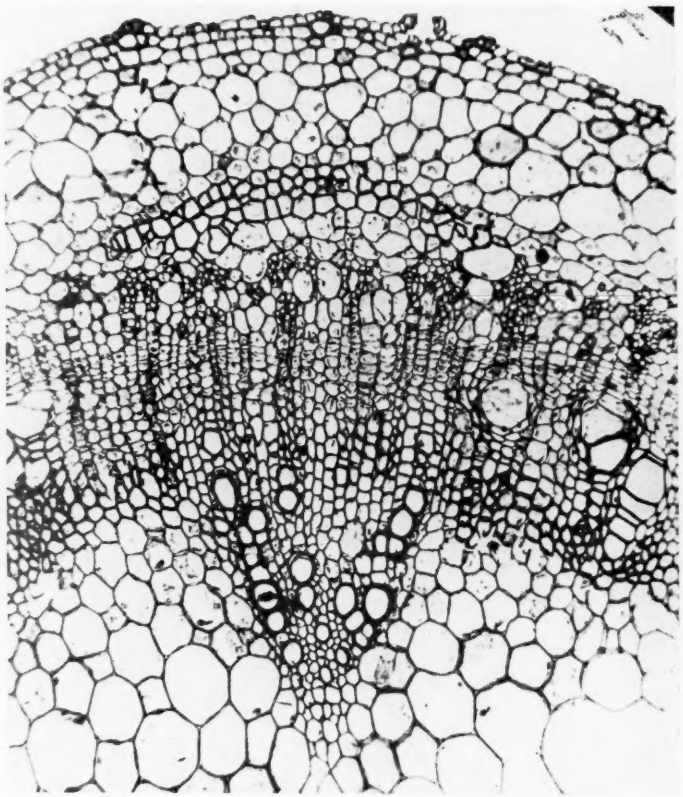

FIG. 14.—Transverse section of mid-region of first internode of plant of experiment II 8 days after first spraying primary leaves with lanolin emulsion.

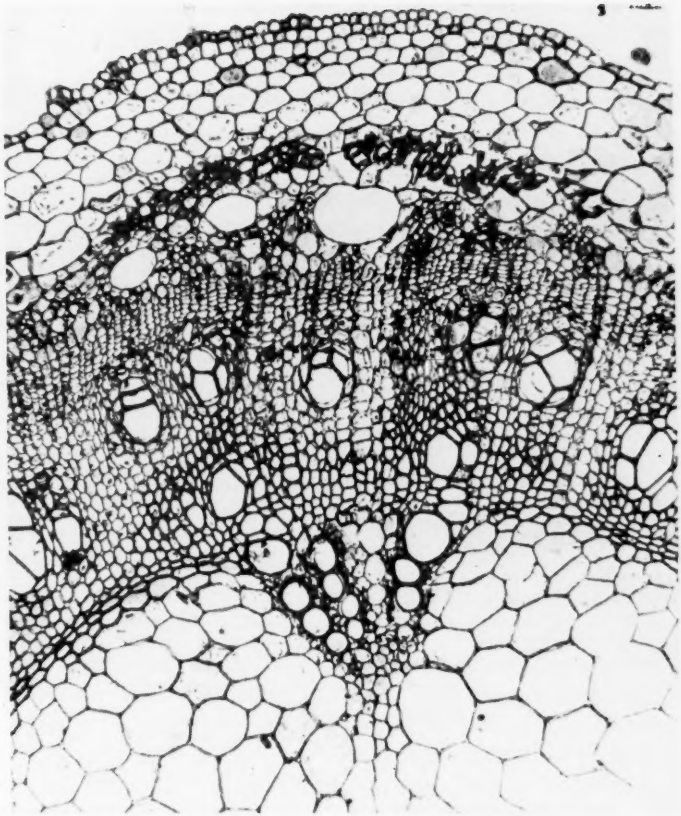

FIG. 15.—Transverse section of mid-region of first internode of plant of experiment II 8 days after first spraying primary leaves with lanolin-alpha naphthalene acetamide emulsion. Relatively greater development of secondary xylem and wall thickening as compared with plant sprayed with lanolin emulsion only is even more pronounced than in the hypocotyl.

lowing fractions: first internode, second internode, primary leaves, and axillary shoots. The fresh weight of corresponding parts from 200 plants of each treatment was recorded and the dry weights determined after drying in a well ventilated oven at 80° C. (table 1).

It is evident from the data of table 1, as well as from the histological evidence, that alpha naphthalene acetamide resulted in the mobilization of solid substances toward the place of application, but that this effect is of less magnitude than that which resulted when

TABLE 1

WET AND DRY WEIGHTS OF PARTS OF PLANTS TREATED WITH 2 PER CENT PASTES OF LANOLIN AND ALPHA NAPHTHALENE ACETAMIDE, LANOLIN AND INDOLEACETIC ACID, AND PURE LANOLIN, AS COMPARED WITH WEIGHT OF CORRESPONDING PARTS OF UNTREATED PLANTS. FIGURES REPRESENT GRAMS WEIGHT OF PARTS FROM 200 PLANTS

Treatment	Axillary shoots		Second internode		First internode		Primary leaves
	Wet weight	Dry weight	Wet weight	Dry weight	Wet weight	Dry weight	Wet weight
Untreated	535.5	58.1	17.0	3.7	69.0	15.0	572.0
Lanolin	525.0	57.0	19.0	4.2	70.0	15.6	569.0
Lanolin and alpha naphthalene acetamide	372.0	41.2	31.0	8.1	82.0	20.0	650.0
Lanolin and indoleacetic acid	328.5	32.4	53.8	13.1	82.8	20.0	607.0

indoleacetic acid was applied in the same percentage composition in lanolin mixtures. As already suggested, a direct comparison of the magnitude of the responses resulting from the use of naphthalene acetamide and those resulting from the use of indoleacetic acid cannot be made unless the possible differences in the relative solubilities of the two substances in lanolin or the liquids in the plant, or in their capacity to move through the plant, is taken into account. Various experiments designed to determine some of these points will be reported in the future.

EXPERIMENT II

Several hundred bean seeds were planted in rows in soil on a greenhouse bench. At the end of the following week the plants were approximately 8 cm. above the soil, the primary leaves averaged 4 cm.

in length, but the second internodes had not yet elongated. Plants in the first row were sprayed with a lanolin emulsion (4) containing 300 mg. of alpha naphthalene acetamide per liter; those in the second row with lanolin emulsion without the acetamide; those of the third row were left untreated. These treatments were replicated twelve times in the same order so that the various treatments occurred evenly throughout the entire length of the bench.

TABLE 2

FRESH AND DRY WEIGHTS AND LENGTH OF DIFFERENT PARTS OF BEAN PLANTS SPRAYED IN SEEDLING STAGE WITH LANOLIN EMULSION CONTAINING 300 MG. ALPHA NAPHTHALENE ACETAMIDE PER LITER AND GROWN FOR 2 WEEKS, AS COMPARED WITH WEIGHTS AND LENGTH OF SIMILAR PARTS OF CONTROL PLANTS OF SAME AGE. WEIGHTS EXPRESSED AS GRAMS PER FIFTY PLANTS

Plant part	Sprayed with alpha naphthalene acetamide-lanolin emulsion			Sprayed with lanolin emulsion			Control plants		
	Wet weight	Dry weight	Length (cm.)	Wet weight	Dry weight	Length (cm.)	Wet weight	Dry weight	Length (cm.)
Hypocotyl	36.7	4.34	5.0	46.8	6.70	5.0	45.6	6.62	5.0
First internode	26.6	3.25	2.5	37.9	5.04	5.0	37.5	5.08	5.0
Primary leaf petiole	35.0	2.37	4.5	36.8	2.43	7.5	37.0	2.53	7.5
Primary leaf blade	240.0	20.0	.....	234.0	21.0	.....	239.0	22.5	.....
Tip	420.0	40.0	.....	1103.0	117.5	.....	1087.0	115.0	.....
Total	758.3	70.0	33.5*	1458.5	152.7	63.0*	1446.1	151.7	63.0*

* Total height of plants above soil level.

Within 24 hours after spraying there was a noticeable difference in the size of the treated and untreated plants. The second internodes, petioles, and blades of the primary leaves of those treated with alpha naphthalene acetamide showed no appreciable increase in length, while corresponding parts of the control plants or those sprayed with emulsion alone showed a definite increase. On the tenth, and again on the twelfth day after planting, the several treatments were repeated on the same plants using fresh emulsions. During this interval the elongation of those sprayed with alpha naphthalene acetamide was less than that of controls; but the above-ground parts, except the second internode, were of greater diameter and were much

stiffer and firmer to the touch. The plants closely resembled those shown in figure 1, but were slightly larger.

Two weeks after the first spraying, the stems of the plants were cut at the soil level and the above-ground portions divided into five parts: hypocotyl, first internode, petiole of primary leaves, blade of primary leaves, and tips (all parts above the second node). The fresh and dry weights of these parts were determined. The height and total leaf area of treated plants were appreciably less than those of untreated plants. The primary leaves of treated plants were much smaller than those of controls, but the amount of solid matter accumulated in the petioles and blades of these leaves during the experiment was approximately the same. Table 2 shows that plants which were treated with alpha naphthalene acetamide accumulated less solid matter in all the parts measured, and the histological evidence (figs. 9–15) shows that this material was distributed in a very different manner in the two cases, much of it apparently being represented in wall thickenings and as secondary xylem.

Additional experiments are being conducted to determine more precisely what substances are mobilized (and to what extent) within plants treated in various ways with alpha naphthalene acetamide, as has already been done with indoleacetic acid (**6, 7, 8, 9**). The histological changes and physiological reactions following the application of either are in a few respects similar, but in many other ways these responses are widely different, so that through the use of these compounds the opportunity to study the differential response and development of cells and tissues in relation to growth substances of known chemical composition is appreciably increased.

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EFFECTIVENESS OF SEVERAL GROWTH SUBSTANCES ON PARTHENOCARPY IN HOLLY

F. E. GARDNER AND PAUL C. MARTH

An earlier paper (3) gave the results of experiments on inducing parthenocarpy in American holly, *Ilex opaca*, by spraying the open blossoms with dilute aqueous solutions of growth substances. Data were presented as to the relative potency of four compounds, indoleacetic, indolepropionic, indolebutyric, and naphthaleneacetic acids, the last named being very much more effective than any of the other three. Recently several other substances have been similarly tried on a comparative basis, and the results, of seeming interest because of their variance in some cases from other reported evaluations of relative effectiveness in plant response, are presented here with the hope that they may eventually help in piecing together the picture of how and why the hormone-like substances bring about the numerous and varied responses associated with their application.

The methods used were essentially those previously reported. The growth substances[1] were dissolved in distilled water and sprayed on the open flowers by means of an atomizer. The tests were applied to small potted plants flowering in the greenhouse and to flowering branches on trees growing on the grounds of the United States Horticultural Station, Beltsville, Maryland.

In table 1 are presented some preliminary results with several compounds of naphthalene on young holly plants flowering in the greenhouse. These plants were found to vary considerably in their ability to set parthenocarpic fruit, depending on their vigor, nutrition, and previous treatment. While only plants selected for their uniformity were used in these tests, yet since 100 per cent setting of fruit was not obtained with treatments which in previous trials had given complete setting, the comparisons were repeated under more uniform conditions. Accordingly the data in table 2 show repetition

[1] Phenylacetic acid was secured from Merck & Company; sodium naphthol(4)-sulphonate from the Eastman Kodak Company; all other naphthalene derivatives from the American Chemical Paint Company, Ambler, Pennsylvania.

of these same compounds and include several others. For these data most of the treatments were applied on branches of a single large

TABLE 1

PERCENTAGE FRUIT SET INDUCED BY SPRAYING HOLLY BLOSSOMS IN THE GREENHOUSE

GROWTH SUBSTANCE	PERCENTAGE CONCENTRATION					
	0.01		0.005		0.001	
	NO. OF FLOWERS	PERCENTAGE SET	NO. OF FLOWERS	PERCENTAGE SET	NO. OF FLOWERS	PERCENTAGE SET
Naphthaleneacetic acid	143	89.0	80	46.6	87	3.5
K-naphthalene acetate	80	12.5	88	12.5	78	0
Naphthalene acetamide	92	98.9	62	90.2	65	36.9
Naphthalene propionic acid*	.....	.....	52	38.6	55	0

* Solubility approximately 0.003 at room temperature.

TABLE 2

PERCENTAGE FRUIT SET INDUCED BY SPRAYING HOLLY BLOSSOMS ON OUTDOOR TREES

GROWTH SUBSTANCE	PERCENTAGE CONCENTRATION					
	0.01		0.005		0.001	
	NO. OF FLOWERS	PERCENTAGE SET	NO. OF FLOWERS	PERCENTAGE SET	NO. OF FLOWERS	PERCENTAGE SET
Naphthaleneacetic acid	213	85.0	165	64.8	176	17.0
K-naphthalene acetate	308	16.2	179	0	181	0
Naphthalene acetamide	294	89.4	121	87.6	182	21.4
Naphthalene thioacetamide	191	9.4	173	10.4	145	0
Phenylacetic acid	226	0	177	0	124	0
Na-naphthol(4)sulphonate	215	0	139	0	167	0
Naphthalenepropionic acid*	.....	.....	237	0	385	0

* Solubility approximately 0.003 at room temperature.

tree, using three branches for each concentration of each compound. It was necessary in some of the treatments to include several smaller trees, although in such cases comparable concentrations of each compound were grouped on the same tree.

The use of trees outdoors necessitated protecting the flowers against pollination by insect or other means. Large glassine bags were fastened over the branches just prior to blossoming. Six days later the bags were removed long enough to count and spray the opened flowers and to remove all unopened buds. The bags were then replaced until all danger of fertilization had passed.

Naphthaleneacetic acid, shown in previous work to be very potent in effecting parthenocarpy in holly, was included in the tests of both greenhouse and outdoor trees. In both cases, and in the three concentrations used, it was somewhat excelled by naphthalene acetamide. The acetamide, in addition to being more potent, has the advantage of not producing any observable nastic disturbances of leaves or petioles. We have often observed the comparative inactivity of this compound in nastic responses with a number of plants.

Of particular interest is the relative ineffectiveness of the potassium salt of naphthaleneacetic acid. Although it brought about an initial swelling of the holly ovaries, the color of the ovaries and of the pedicles turned progressively yellow instead of acquiring the deep green hue of those treated with naphthaleneacetic acid, and most of the young fruits soon abscissed. At present no explanation is offered for the low activity in parthenocarpy of the potassium salt which, in various other plant responses, has been reported of high activity. The difference in pH between a solution of the acid and of the potassium salt and the probable resulting difference in cell penetration, however, should be considered in view of the work of ALBAUM, KAISER, and NESTLER (1). They showed that the penetration of indoleacetic acid into *Nitella* cells takes place more rapidly at low pH values of the external medium than at high ones, and attributed the results to the effect of high hydrogen ion concentration depressing the dissociation of indoleacetic acid which, evidence indicates, enters the cells in the non-dissociated form. It seems hardly necessary to state that the greater activity of naphthaleneacetic acid than of the potassium salt was not due to its acidic nature causing release of naturally occurring bound auxin. Possibly it should be recorded that acetic acid, in a range of concentrations, did not stimulate parthenocarpy when sprayed on holly flowers.

In view of the high activity of naphthalene acetamide, it is curious

that its thio compound should be of such low effectiveness. No data are available on the relative dissociation of these compounds which might throw light on the reason for their different effectiveness.

Phenylacetic acid, while not rated high in activity in other plant responses, gave no indication whatever of any stimulation in these tests. This was also the case with sodium naphthol(4)sulphonate, which was included because it is one of the compounds reported by TRAUB (**4**) to have a marked stimulating effect on rooting of *Passiflora* and *Bignonia* cuttings, but to be without effect on *Avena* curvature.

With respect to the nature of the action of these substances in stimulating fruit development, little can be said. It appears that they prevent the formation of an abscission layer at the base of the flower pedicle, thus permitting the flow of nutrients necessary for growth of the fruit and perhaps substituting in part for the stimulus set up by the developing fertilized ovule. At least the subsequent histological development of the parthenocarpic fruits has been shown to parallel that of normal, pollinated ones (**2**).

As more data become available on the effectiveness of many compounds in various plant responses, such as curvatures of stem or leaf, elongation, root production, gall formation, parthenocarpy, etc., it becomes increasingly evident that the order of effectiveness in one response may be very different in terms of some other response, and that "relative activity" of a compound is without meaning except with reference to the particular test method and probably also to the particular plant used.

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ADOLF CARL NOÉ

In the death of Dr. Adolf Carl Noé on April 10, 1939, American geology and paleobotany have lost a devoted leader. Dr. Noé was born in Austria on October 28, 1873, and educated in part at the universities of Gratz and Göttingen. During his student days at Gratz he developed an interest in paleobotany, and was a demonstrator in paleobotany in that institution from 1895 to 1897. On coming to America, he filled several positions in the teaching of modern languages and literature. He received an A.B. degree at Chicago in 1900, and the Ph.D. in Germanic Languages and Literatures in 1905. He was a member of the staff of this Department until 1923. In that year he transferred to the Departments of Geology and Botany, where he remained for the rest of his life. He came into contact with many students in these departments during these years.

In the field of paleobotany he distinguished himself by discoveries of coal balls in the American coal fields, by becoming one of the most active and enthusiastic collectors of paleobotanical material, and by developing new methods of studying paleobotanical materials, especially refinement of the nitrocellulose peel method of making microscopic sections of coal balls.

Dr. Noé was associated with various state geological surveys, particularly in Illinois, Iowa, and Kentucky. Among his scientific contributions were several books: *The Fossil Flora of Northern Illinois; Ferns, Fossils, and Fuels;* a textbook on coal, and numerous scientific papers. He was a member of many learned societies here and abroad, and was honored and decorated for his scientific and humanitarian services after the World War. He was a man of kindly spirit, with a keen sense of humor. He is much missed by his students and colleagues.

E. J. Kraus

BRIEFER ARTICLE

POLYEMBRYONY IN MYRCIARIA CAULIFLORA

(WITH ONE FIGURE)

The jaboticaba, *Myrciaria cauliflora*, was introduced to this country from Brazil a few decades ago, and in more recent years trees of it have fruited in Florida. This may be a promising new fruit crop for Florida and possibly also for other subtropical regions of continental United States.

Dissection of twenty-five sprouted seeds has revealed that polyembryony is usually encountered. In the material used the number of seedlings of varying sizes from single seeds ranged from one to six; the usual number was two. When more than three embryos were present in a single seed, some of them were tiny. Figure 1 (p. 234) shows one, two, and three seedlings sprouting from single seeds. Under the usual nursery conditions only the larger seedlings survive from a single seed, usually only two.

Morphological studies to trace the origin of the supernumerary embryos are under way.—HAMILTON P. TRAUB, *Division of Fruit & Vegetable Crops and Diseases, U.S. Department of Agriculture, Orlando, Florida.*

FIG. 1.—Sprouting seeds of *Myrciaria cauliflora*, showing one, two, and three sprouting embryos per seed.

CURRENT LITERATURE

Le genre Mycena. Étude cytologique et systématique des espèces d'Europe et d'Amérique du Nord. By ROBERT KÜHNER. Encyclopédie mycologique X. Paris: Paul Lechevalier, 1938. Pp. 710. Illustrated. 300 fr.

According to the author, about one-sixteenth of the agarics of Europe are included in the genus *Mycena*, and it may be assumed that in temperate North America the proportion is substantially the same. The fructifications of the majority of the species are small and ephemeral, and the descriptions of the older mycologists emphasize to too great an extent superficial and often essentially secondary or inconstant characters. These facts have combined to make this one of the most difficult genera of the Agaricaceae from the taxonomic point of view. In the present volume an attempt is made to consider all European species and as many North American species as possible, using what is largely a new arrangement based primarily upon the microchemical reaction of the spores and hyphae to iodine, and stressing such microscopic features as cystidia and the surface cells of pileus and stipe. Where the original descriptions are inadequate and authentic specimens have not been available, it has been impossible to fit the species concerned into the author's system; but full notes, including translations of the original descriptions, are given for all such forms. Seventeen species, three varieties, and eight forms are described as new, several of them being attributed jointly or solely to R. MAIRE, whose notes were turned over to the author. Considering the scope of the work, this would seem to suggest a conservative approach.

While mainly taxonomic, nearly a quarter of the text is concerned with more general matters, including technique, anatomy, morphology, and cytology. Much of this, particularly the discussion of so-called sexual phenomena, is of general interest.

Only experience can determine the extent to which the taxonomic subdivisions proposed by the author will prove to be applicable to the North American species, but the detailed original studies and the careful compilation of previous information make this volume a new starting point for the study of the genus directly concerned and furnish abundant suggestion for a similar approach to other genera of the Hymenomycetes.—G. W. MARTIN.

The British Islands and Their Vegetation. By A. G. TANSLEY. Cambridge, England: University Press; New York: Macmillan Co., 1939. Pp. xxxviii+930. Illustrated. 45/.

Ecologists and all interested in the natural and semi-natural vegetation of the British Islands should be delighted with this masterly and comprehensive description by the one best qualified to present it. The book is a modern out-

growth of the earlier volume, *Types of British Vegetation*, published in 1911, which did much to stimulate the research embodied in many of the papers on British plant communities appearing in the *Journal of Ecology* over the last quarter of a century, upon which the present volume draws very largely. Professor TANSLEY'S purpose, admirably fulfilled, has been to weld this material "into a continuous story which can be read, and not merely "consulted," by the student—British or foreign—who wants trustworthy information about the British plant communities, but who is not an ecological specialist." The resulting treatment is therefore somewhat longer, more explanatory of concepts and terms, and more repetitious than if intended primarily for ecologists. There are very few of the latter, however, excepting those completely unsympathetic with Professor TANSLEY'S ecological philosophy, who will not derive both pleasure and profit from the orderly way in which he has marshalled and integrated the facts (possibly already familiar) in accord with it. The treatment contains little of phytosociological nomenclature (of BRAUN-BLANQUET), or of bioecology, although a chapter on the "biotic factor" is included and many effects produced by animals on plant communities are recorded. It is in complete agreement with the dynamic concepts previously developed by Professor TANSLEY in his books and theoretical papers.

The volume is divided into nine parts: the British Islands as environment of vegetation (146 pp.); history and existing distribution of vegetation (64 pp.); nature and classification of vegetation (30 pp.); the woodlands, excluding plantations of exotic trees (244 pp.); the grasslands, including all permanent planted pastures of native species as well as "natural" grassland formed from grazing other vegetation types (92 pp.); the hydroseres, including freshwater, marsh, fen, and bog vegetation (144 pp.); heath and moor (52 pp.); mountain vegetation (41 pp.); and maritime and submaritime vegetation, excluding intertidal seaweed communities on rocky coasts but including salt-marsh (89 pp.). There is an understandable prominence of data from southeastern England, where many of the modern detailed studies have been made.

An adequate index is provided, and pertinent references follow each of the forty-three chapters. The book is graphically and beautifully illustrated by 179 text figures and 162 plates containing 418 photographs. It should be required reading for all serious students of ecology anywhere, and for any American botanists who feel that the present descriptions and summaries of the vegetation of the United States or any of its larger subdivisions are at all adequate or complete.—C. E. OLMSTED.

German-English Botanical Terminology. By HELEN and ERIC ASHBY, HAROLD RICHTER, and JOHANNES BÄRNER. London: Thomas Murby & Co., 1938. Pp. xi+195. 10/.

The reader will find much that is fundamental in botany as well as achieve a vocabulary and a method of expression in German from the use of this novel

and excellent book. Textual material reads smoothly, connectedly, and easily in English on one page and on the facing page there is as nearly a literal translation in German as is feasible, considering the difference in method of expression and phraseology of the two languages. Chapters deal with morphology, phylogeny, cytology and genetics, physiology, ecology, and pathology in a gratifyingly competent way, considering the brevity of the book. There are also appendixes giving the common names of wild and cultivated plants, names of the more important plant diseases, abbreviations used in English and German botanical literature, and an index in each language. The book does not take the place of an extensive dictionary, but it does far more than any dictionary can by giving the flow and sense of written language which a reader may desire to interpret.—E. J. KRAUS.

Clés des Mucorinées. By N. A. NAUMOV. Encyclopédie mycologique IX. Paris: Paul Lechevalier, 1939. Pp. xxxvi+137. Illustrated. 100 fr.

Translated from the second Russian edition by S. BUCHET and I. MOURAVIEV, the French text incorporates additional material contributed by the author. A brief introduction discusses the morphology of the fungi constituting the order, but the bulk of the volume is in the form of a detailed key to the suborders, families, genera and (under each genus) to the species recognized. A very complete index lists, together with recognized genera and species, synonyms and rejected names not mentioned in the text. Since H. ZYCHA's monograph (*Kryptogamenflora der Mark Brandenburg* VIa, 1935) appeared almost simultaneously with the Russian text, an appendix is devoted to a consideration of the divergences between the two treatments. NAUMOV does not include the Endogonaceae, but even so recognizes approximately half as many species again as does ZYCHA. One new genus, *Protoabsidia*, and a number of new species are indicated, without Latin diagnoses. The striking American genus *Mycotypha*, published in 1932, has not yet found its way into European monographs.

While the present work will not replace ZYCHA's excellent and logical treatise, it constitutes a valuable supplement to it. Together they should provide needed impetus to the study of these highly significant and important fungi.—G. W. MARTIN.

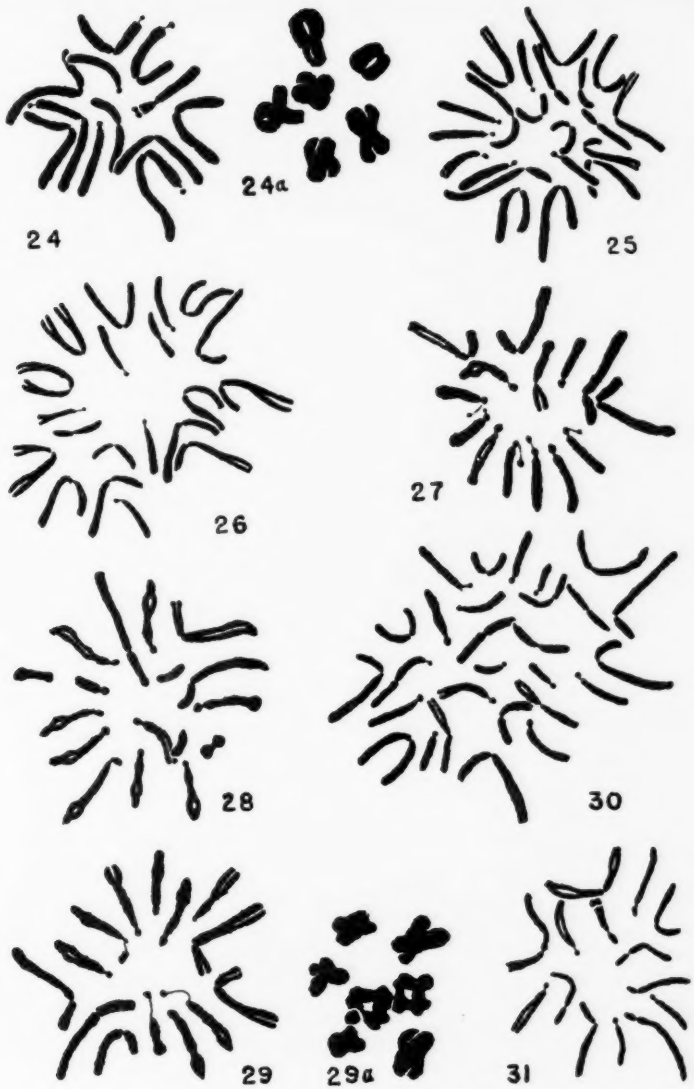
Introduction to Floral Mechanism. By S. G. JONES. Glasgow: Blackie & Son, 1939. Pp. xi+274. Figs. 71.

A generalized presentation of floral morphology, cytology, genetics, and plant breeding designed to acquaint the elementary student with these subjects. The text is direct, largely descriptive, and presents much detail, but in some instances the morphological homologies are not clearly drawn nor critically stated. The illustrations are numerous, well drawn, and though lacking in many details essential to critical morphological studies and interpretation, no doubt serve the purpose intended for the elementary student.—E. J. KRAUS.

Bibliography of References to the Literature on the Minor Elements and their Relation to Plant and Animal Nutrition. Originally compiled by L. G. WILLIS. Chilean Nitrate Educational Bureau, Inc., 120 Broadway, N.Y., 1939. Pp. 488. $1.00.

The third edition of this valuable annotated bibliography contains 4628 abstracts dealing with the elements used and needed in small amounts in the nutrition of plants, animals, and man. The number of authors abstracted is about 2200. The elements are arranged in alphabetic order, and the authors also alphabetically under each element included. It is interesting to note that 53 of the 92 naturally occurring elements are listed in the index. Not all of these are certainly required by any one species of plant or animal, nor is the inclusion of an element in the list to be construed as indicating usefulness. If they have been investigated, they are included in the list.

The first edition, published in 1935, included just over 1800 abstracts; and the second edition in 1937, almost 2800. These earlier editions were less complete than the present one, nevertheless the increasing number of citations is an index of the extraordinary interest in the trace elements at the present time. The activity in the field will undoubtedly continue, since we know very little yet of the physiological functions of any of these elements. The Chilean Nitrate Educational Bureau deserves commendation for the enlightened policy which established and maintains a publication so vital to research on plant nutrition. Every student of nutrition will find this vast storehouse of information an indispensable adjunct to his work.—C. A. SHULL.



BEAL—CALOCHORTUS

Corrected reproduction, to be substituted for the incorrect one shown on p. 538 of the issue of March, 1939.